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# GROWTH AND SEXUAL MATURITY IN BRAHMA AND LEGHORN FOWL[1]

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Evidence has been offered by Hays (3) that early sexual maturity in Rhode Island Red fowls is dependent in part on two dominant genes, one of which is sex-linked. Hays' hypothesis suggests that the presence of either or both of these dominant genes will produce a bird that normally begins to lay at the age of 215 days or earlier. Warren (11) also finds some evidence of a sex-linked gene for early sexual maturity in a cross between White Leghorns and Rhode Island Reds. This bulletin offers additional information on the inheritance of time of sexual maturity based on a cross between a single comb White Leghorn and Light Brahma fowl.

It is evident that early sexual maturity in the domestic fowl is a desirable character, and that, other things being equal, early maturing pullets will produce in their first laying year a larger number of eggs than late maturing pullets. But it is possible to over-emphasize the importance of early sexual maturity, for Graham (2) finds that early maturing birds weigh less than late maturing birds on attaining sexual maturity. Graham does not state whether the ultimate weight of the early maturing bird is greater or less than that of late maturing birds. Jull (5), using Barred Plymouth Rocks, found that a significant positive correlation exists between the mean weight of the first ten eggs laid in the pullet year and time to sexual maturity. This correlation is shown to be important by the observations of Maw and Maw (7), who find that the earlier in life laying commences, the longer is the time required to attain production of eggs of standard size. Further, these same authors have shown that the mean annual egg weight of early maturing pullets is less than that of the late maturing pullets. It would seem, then, that if birds mature too early, it may result in small body size and small egg size. But very little experimental evidence has been produced to show whether body size or egg size is permanently retarded by early maturity.

Marble and Hall (6) have found in breeding for egg production in high-line and low-line Leghorns a very interesting relation between the first, or pullet, year average body weight and the number of days to sexual maturity. They present curvilinear trends showing the increase in body weight from the years 1908 to 1927 for birds of the high-lines and from the

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Part of this study was made at the Bussey Institution while the author was serving as research assistant to Professor W. E. Castle, under the joint auspices of The Carnegie Institution of Washington and of Harvard University. The records on which this study is based were collected at the Agricultural Experiment Station of the Rhode Island State College and permission for publication was granted by the Director of that station.

[2] Symbols used throughout the text: B = Brahma, L = Leghorn, B x L = Brahma male mated with Leghorn female, etc. In all cases the male parent is indicated first.

years 1913 to 1927 for birds of the low-lines. Curvilinear trends are also shown for the number of days to sexual maturity for both the high and low lines. There has been, according to their observations, a steady decrease in the number of days to sexual maturity for the high-lines from 1908 to 1927. The low-lines also show a decrease in number of days to sexual maturity, though not so pronounced as that found in the high-lines. Further, both the high-lines for a twenty-year period and the low-lines for a fifteen-year period show an increase in the mean weight of egg laid during the pullet year.

The work of Marble and Hall suggests that the number of days to sexual maturity may be reduced materially without decreasing the ultimate body weight of the bird or its egg weight, if rigid selection is practiced. On the other hand, the apparent acceleration of maturity in high and low lines alike may depend merely upon improved management, particularly feeding.

In animal husbandry it is generally recognized that growth is retarded if animals are bred at too young an age, and in certain cases the animal may be, in consequence, somewhat smaller as an adult. It has been pointed out by Mumford (8), however, that in swine the difference in size at maturity between animals bred at a very young age and those bred at a later stage is insignificant. Reed et al. (10) state that, in Holstein cattle, cows bred to calve at 24 months of age did not develop so well as the animal on the same feed bred to calve at 30 months of age. Withycombe et al. (13) show that Hereford cows producing a calf each year at the ages of two, three, and four years, averaged about 100 lbs. less when five years old than cows which produced calves only at ages three and four years. From the foregoing facts, it is apparent that animals which suckle their young, if bred at too early an age, have their growth delayed or inhibited, but it is not altogether clear that the size ultimately attained will be limited by such early breeding.

Pease (9), while studying the inheritance of weight in a Polish-Flemish cross of rabbits, was able to make certain observations on date of sexual maturity of these two breeds. His results indicate that time of sexual maturity is not affected by sex and probably not by litter size. Pease further concludes that there is a considerable degree of association between maximum adult weight and slow maturity in rabbits, but that this relation is conspicuously absent in many individuals.

Castle (1) crossed a race of large rabbits with a race of small rabbits. Sexual maturity in the small race occurs at approximately 160-180 days. Sexual maturity occurs later in the large race—about 200 to 250 days—and, as Castle states, "less obviously affects the character of the growth curve." The curve for the hybrid females flattens out abruptly at the time of sexual maturity, which comes in this group somewhat later than in the small race but much earlier than in the large race. It is interesting to note that the growth curves of both sexes flatten out at the time of sexual maturity.

The relationship between growth and time of sexual maturity in the domestic fowl has received little attention from poultry investigators, and it seems desirable, therefore, to observe the effect of this factor on the ultimate size of the bird. Birds being oviparous and mammals viviparous, it is possible that time of sexual maturity has not the same significance in both. Yet intense egg production is a heavy drain on the growing bird,

as are gestation and lactation on a mammal, and we might reasonably expect to find similar effects on the mother in both cases.

## MATERIALS AND METHODS

Information on the source and nature of the birds employed in this investigation has been presented in Bulletin 228 of the Rhode Island Agricultural Experiment Station, together with a description of the experimental methods used. A cross was made between single-comb White Leghorns and Light Brahmas. Reciprocal $F_1$ and $F_2$ generations were obtained. Individual pedigree records were kept for every bird used in the experiment. Each chick was weighed at hatching time and thereafter at intervals of one week during the first three months and at monthly intervals thereafter until maximum weight was attained. The age at first egg was used as the age at sexual maturity. It was not practical to weigh each bird exactly on the day of sexual maturity, and in many instances it was necessary to estimate this weight by interpolation between the last weight taken prior to laying of the first egg and the first weight taken subsequently.

The Leghorn is a small breed, the female weighing approximately 1,600 grams and the male 2,000 grams at 10 months of age. The Brahma is one of the largest breeds of domestic fowl. The Brahma female weighs approximately 3,200 grams and the male 4,000 grams at 10 months of age. Brahma individuals are thus just about twice as heavy as Leghorns.

The numbers of parents and crossbreds used in this experiment were Brahma 61, Leghorn 374, $F_1$332, $F_2$260, and backcross 34.

## SEXUAL MATURITY

A graphic presentation of the variation in age at first egg for the Leghorns, Brahmas, and their $F_1$ and $F_2$ reciprocal hybrids is given in figure 1. The average age at first egg is 200 days for the Leghorns and for the Brahmas 291 days. The Leghorns show a distribution for age at sexual maturity from 120 days to 310 days with a principal modal group from 200 to 210 days, but with a secondary modal group between 170 and 180 days. As shown below, this bimodal condition is probably due to combining data obtained in different seasons or under different environmental conditions. The Brahmas show a distribution for age at first egg ranging from 210 to 390 days and may accordingly be classified as a late maturing breed.

Figure 2 shows the distribution of the Leghorns for the years 1921 to 1926. The mean number of days to first egg varies somewhat from year to year, but on the whole there is a general decrease following the year 1922. Inasmuch as there was no selection for early maturity, this decrease may be attributed to environmental differences in which better feeding methods probably played a part. A recombination of the data excluding those of 1925 showed that the secondary mode at 180 days in the combined data is due wholly to the birds reared in 1925. It was not found in the flocks of the four earlier years or in the 1926 flock.

In general the age at first egg for the reciprocal hybrids, as shown in figure 1, approaches that of the earlier maturing parent. The average age at first egg, based on all matings for the years 1923 to 1926, shows a difference between the reciprocal crosses which is statistically significant. The female from the cross of Brahma male with Leghorn female mature,

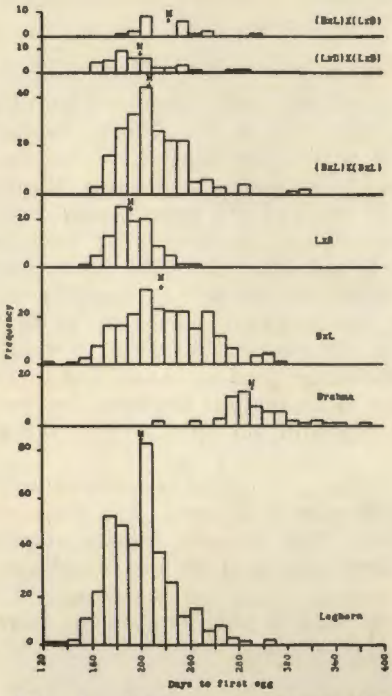


Fig. 1. Frequency polygons showing variation in age at first egg for Brahmas, Leghorns, and their $F_1$ and $F_2$ hybrid progenies.

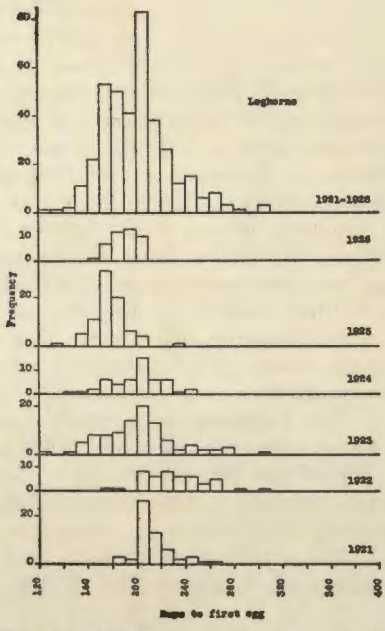


Fig. 2. Frequency polygons showing variation in age at first egg for Leghorns in the years 1921 to 1926.

on the average, at 219 days. The females from the cross of Leghorn male with Brahma female mature earlier, at 193 days (see table 1).

It would seem that a sex-linked gene or genes determines in part, the difference between Leghorns and other breeds as regards age at the production of the first egg. Figure 3 shows the frequency distribution of days to sexual maturity for the $F_1$ reciprocal hybrids year by year from 1923 to 1926 inclusive. The B x $L^a$ individuals for the years 1923, 1924, and 1926 are, on the average, phenotypically later maturing than the individuals hatched in the year 1925. There is no evidence of the action of a sex-linked gene in the B x L population for the year 1925, while the B x L populations for the years 1923, 1924, and 1926, when compared with the L x B populations, show a mean difference at age of first egg which is statistically significant. The L x B individuals are nearly all early maturing with but very little variation from year to year. The variation in days to sexual maturity for the B x L hybrids for the various years, however, is no greater than that found in the Leghorns for the same years.

For age at first egg the 1925 data, both for the Leghorn and the B x L birds, are exceptional. The mean age at first egg for the 1925 Leghorns is nearly 30 days earlier than that of the Leghorns hatched during the years 1923, 1924, and 1926. Further, the mean age at first egg for the 1925 B x L birds is nearly 40 days earlier than that of the B x L birds hatched during the years 1923, 1924, and 1926. This exceptionally early

TABLE 1. *Statistics on Brahma and Leghorn Fowl and their $F_1$ and $F_2$ hybrids*

| | Weight at first egg | | | | Maximum adult weight | | | Days to first egg | | | Percentage of adult weight at first egg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of birds | Mean | σ | C.V. | Mean | σ | C.V. | Mean | σ | C.V. | Mean |
| Leghorn | 374 | 1553± 6 | 185 | 11.9 | 1697± 7 | 202 | 12.0 | 200±1 | 27 | 13.6 | 91 |
| Brahma | 61 | 3133±24 | 382 | 12.2 | 3190±32 | 375 | 11.8 | 291±3 | 29 | 10.2 | 98 |
| B x L | 233 | 2179±12 | 278 | 12.8 | 2332±12 | 272 | 11.7 | 219±2 | 34 | 15.8 | 93 |
| L x B | 99 | 2200±18 | 273 | 12.4 | 2445±22 | 321 | 13.2 | 193±1 | 16 | 8.5 | 90 |
| (BxL) x (BxL) | 194 | 2275±16 | 339 | 14.9 | 2521±19 | 400 | 15.9 | 208±1 | 28 | 13.6 | 90 |
| (LxB) x (LxB) | 40 | 2324±30 | 284 | 12.2 | 2615±37 | 351 | 13.4 | 200±3 | 28 | 14.1 | 89 |
| (BxL) x (LxB) | 26 | 2262±45 | 341 | 15.1 | 2508±43 | 326 | 13.0 | 223±3 | 25 | 11.5 | 90 |

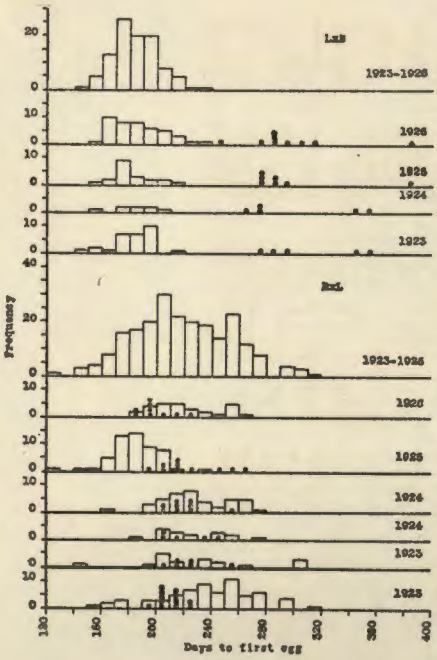


Fig. 3. Large dots show the age at first egg of the 41 Leghorn and 25 Brahma females of the $P_1$ generation. The polygons show the variation in age at first egg of their $F_1$ hybrid daughters in the years indicated. The combined data for the $F_1$ daughters in the years 1923-1926 are also shown.

maturity of the birds hatched in the year 1925 may reasonably be attributed to better environmental conditions.

The $F_2$ females from the cross B x L matured at 208 days, and the $F_2$ females from the cross L x B matured at 200 days, while the $F_2$ females from the cross of (BxL) x (LxB) matured at 223 days. The differences between the variability of the $F_1$ and $F_2$ hybrids are not statistically significant.

There is no clear evidence of the action of a sex-linked gene in any of the $F_2$ samples. It may also be pointed out that the action of a dominant autosomal gene, as proposed by Hays (3), is not evident in the $F_2$ samples. For if one dominant autosomal gene were responsible for the difference between the late maturing Brahma and the early maturing Leghorn, then the reciprocal hybrids would be all early maturing, and the $F_2$ samples would show a bimodal distribution, which is not the case.

It has been previously pointed out that for the Leghorns the number of days to first egg ranges from 120 to 310 days. It is essential to establish, if possible, the genetic constitution of those Leghorns which, phenotypically, are early maturing, and also of those which are late maturing. This comparison is obtained by mating each group to a Brahma male. To this end six matings were studied involving six different Brahma males and 41 Leghorn females. Figure 3 shows the variation in age at first egg of these 41 Leghorn females and also of their $F_1$ daughters. More than half of the 41 Leghorn females matured later than 215 days (the age arbitrarily chosen by Hays as the division between early and late maturing birds). It is possible that some of these late maturing Leghorn females were delayed in laying their first egg because of adverse environmental conditions. However, they were not all hatched in the same year, and it is not probable that the environment was entirely responsible for their late maturity.

By applying Fisher's method of analysis of variance, it is possible to estimate whether the progeny from late maturing Leghorns differs significantly from the progeny of early maturing Leghorns, when both groups are mated to the same late maturing Brahma male, (see table 2.) There is no significant difference in days to sexual maturity between the progenies within any one of these matings. The progenies of late maturing Leghorns do not differ significantly from the progenies of early maturing Leghorns, when both groups are mated to the same late maturing Brahma

TABLE 2. *Analysis of variance in age at first egg among the progeny of various males, each mated to several females*

| | $F_1$1923 (♂ B1R) | | | $F_1$1923 (♂ B2C) | | | $F_1$1924 (♂ B11B) | | |
|---|---|---|---|---|---|---|---|---|---|
| | D/F | Sum of squares | Mean square | D/F | Sum of squares | Mean square | D/F | Sum of squares | Mean square |
| Between progeny of different hens | 6 | 9,946 | 1,657 | 4 | 2,024 | 506 | 3 | 3,454 | 1,151 |
| Within progeny of the same hen | 58 | 65,191 | 1,124 | 13 | 26,015 | 2,099 | 12 | 2,958 | 247 |
| Total | 64 | 75,137 | 1,174 | 17 | 28,039 | 1,649 | 15 | 6,412 | 427 |
| | $F_1$1924 (♂ B11V) | | | $F_1$1925 (♂ B12F) | | | $F_1$1926 (♂ B34C) | | |
| | D/F | Sum of squares | Mean square | D/F | Sum of squares | Mean square | D/F | Sum of squares | Mean square |
| Between progeny of different hens | 5 | 1,598 | 320 | 9 | 2,300 | 256 | 8 | 8,681 | 1,085 |
| Within progeny of the same hen | 35 | 23,670 | 676 | 46 | 14,470 | 315 | 19 | 8,300 | 437 |
| Total | 40 | 25,268 | 632 | 55 | 16,770 | 305 | 27 | 16,982 | 628 |
| | $F_2$1925 (♂ G24C) | | | $F_2$1925 (♂ G42B) | | | $F_2$1926 (♂ G32G) | | |
| | D/F | Sum of squares | Mean square | D/F | Sum of squares | Mean square | D/F | Sum of squares | Mean square |
| Between progeny of different hens | 7 | 3,293 | 470 | 10 | 11,740 | 1,174 | 6 | 8,819 | 1,469 |
| Within progeny of the same hen | 52 | 2,479 | 477 | 76 | 53,620 | 706 | 25 | 33,626 | 1,345 |
| Total | 59 | 28,079 | 476 | 86 | 65,360 | 760 | 31 | 42,445 | 1,369 |

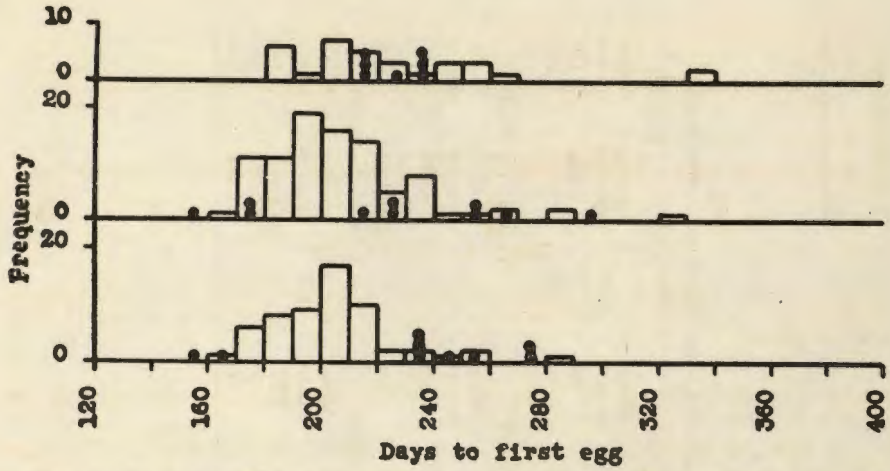


Fig. 4. Large dots show the age at first egg of the $F_1$ mothers; the frequency polygons show the variation in age at first egg of their $F_2$ daughters for each of the three $F_1$ matings.

male. The hens in each mating seem not to differ genetically among themselves with respect to sexual maturity.

Of still further interest is the fact that the three $F_1$ B x L matings give results similar to those found in the $P_1$ matings and there is no significant difference in days to sexual maturity between the progenies within any one of these $F_1$ matings. (see table 2). Figure 4 shows the variation in number of days to first egg for three different matings of $F_1$ females and for their $F_2$ daughters. Over half of these $F_1$ females matured later than 215 days. However, we find no significant difference between the $F_2$ progeny from early maturing and from late maturing $F_1$ parents, when mated to the same male, as shown in table 2. Apparently these $F_1$ females are genetically alike with respect to sexual maturity.

The hypothesis advanced by Hays postulates that early maturity depends upon two pairs of dominant genes, one of which is sex-linked. Presence of either of these genes will result in early maturity,—that is, the birds will lay prior to age 215 days. A cumulative effect for these two genes is suggested as possible. If the assumption is made that the Brahma is genetically late maturing, because it has neither the sex-linked gene E nor the autosomal gene E′, and if the Leghorn is assumed to be early maturing, because it has both of these genes, then with complete dominance the reciprocal hybrids from this cross should be all early maturing, laying their first egg prior to 215 days. But we observe, in figure 1, that the reciprocal hybrids are not all early maturing. In fact, the hybrids from the cross of Brahma male with Leghorn female are over 50 per cent late maturing. It is true that hybrids from the cross of Leghorn male with Brahma female are, for the most part, early maturing. The difference in number of days to first egg between the reciprocal hybrids suggests, indeed, the action of a sex-linked gene. The evidence is in harmony with this idea, except the result for the B x L hybrids in the year 1925. The fact that the B x L hybrids are later maturing than the L x B hybrids, but not so late maturing as the Brahma breed, suggests an effect of the

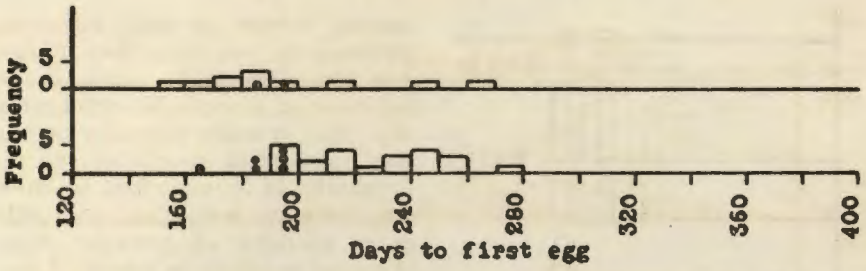


Fig. 5. Large dots show the age at first egg of 2 Leghorn and 6 $F_1$ female parents; the frequency polygons show the variation in age at first egg of their reciprocal backcross daughters.

assumed sex-linked gene E cumulative with the effects of other genes for early maturity, which may be autosomal.

A critical test of the applicability of Hays' hypothesis to this cross is offered in two backcross matings. If we assume the Leghorn, which is early maturing, to have a genetic formula for the male (E)(E)E′E′ and for the female (E)(—)E′E′, and if we assume the Brahma male to have the genetic formula (e)(e)e′e′ and the female (e)(—)e′e′, then we should expect very definite results when these two breeds are crossed together. The $F_1$ reciprocals should all be early maturing, laying their first egg prior to 215 days. When an $F_1$ male from the cross of Brahma male with Leghorn female, having the genetic formula (E)(e)E′e′, is mated with Leghorn females having the genetic formula (E)(—)E′E′, then the expectation is that all the backcross progeny will be early maturing. All but three of the eleven individuals were indeed early maturing. If, now, we backcross to a Leghorn male the $F_1$ females from a cross of Brahma male with Leghorn females, the expectation is that these backcross progeny will also be early maturing. But, referring to figure 5, we observe that over two-thirds of them are actually late maturing.

The results obtained in this cross between Brahma and Leghorn are not in agreement with the hypothesis of Hays, that two pairs of dominant genes are responsible for early maturity. There is some $F_1$ evidence of the action of a sex-linked gene for early maturity, but beyond that the hypothesis is not verified. We have observed also that certain individuals in the Leghorn breed are phenotypically late maturing, while others are early maturing. However, when we mate these early or late maturing birds to the same late maturing Brahma, we obtain no significant difference in age at first egg for the pullets produced by the two classes. It is quite evident that, for the birds used in this cross, age at first egg is not a reliable criterion to use in classifying individuals as genetically early or late maturing. Accordingly, the Hays hypothesis is incapable of proof or disproof from these experimental data.

## THE RELATION OF GROWTH TO SEXUAL MATURITY

It is evident that the Leghorns used in this study as a group mature considerably earlier than the Brahmas, as is generally conceded by poultry breeders for these breeds. However, poultry breeders will also agree that certain strains of Brahmas attain sexual maturity earlier than other strains, but unfortunately there is no experimental evidence available to substantiate this belief. Further, there is no evidence to show that the average

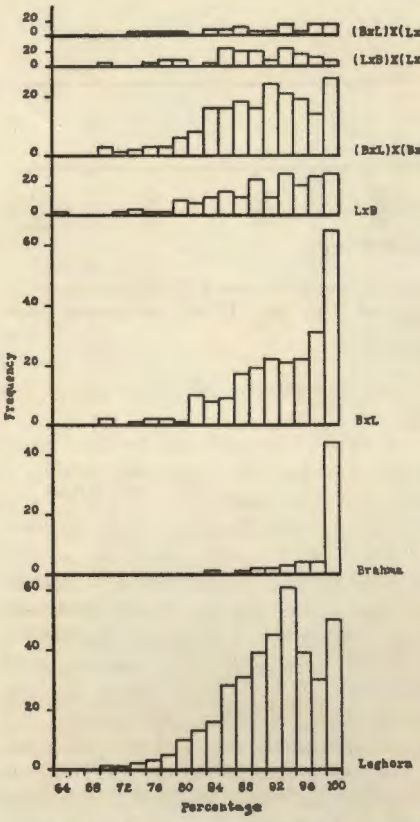


Fig. 6. Frequency polygons showing the variation in percentage of adult weight attained at age of first egg by Brahmas, Leghorns, and their $F_1$ and $F_2$ hybrid progeny.

mature weight of early maturing Brahmas is less than that of late maturing Brahmas. Little or no information is available concerning the effect of early maturity on the maximum adult weight of the individual. It is important to know whether early sexual maturity acts as an inhibitor on growth. Also, if sexual maturity is inherited separately from adult size, then it should be possible to produce a strain of early maturing and a strain of late maturing birds that average the same in maximum adult weight.

The percentage of adult weight attained at age of first egg is shown in figure 6 for the Brahmas, Leghorns, and for their $F_1$ and $F_2$ hybrid offspring. Here we observe that the Leghorns attained an average of 91 per cent o ftheir adult weight at age of first egg, while the Brahmas attained 98 per cent (see table 1). The reciprocal crossbreds all resemble the Leghorn parent in that they produce the first egg before attaining full growth. The B x L hybrids attained 93 per cent of their adult weight, while the L x B hybrids attained 90 per cent of their adult weight when the first egg is produced. The $F_2$ groups are also like the smaller race, having attained approximately 90 per cent of their adult weight at age of first egg.

The percentage of adult body weight at first egg for all groups ranged from 63 to 100 per cent. It may be noted that the grouping of these data is skewed to the right.

It has been pointed out previously Waters (12) in this same Brahma and Leghorn cross that mature size is dependent upon two factors: (1) the rate at which growth takes place and (2) the duration of growth. The practical termination of growth comes at ten months of age for both the breeds and for their hybrids. The initial hatching weight is substantially the same in all groups, and the duration of growth is the same. It follows, therefore, that the differential genetic factors which influence adult weight do so through their effects on growth rate.

The question now is whether genes for sexual maturity influence the adult weight of the individual through their effects on the growth rate. Regardless of the number and nature of the genes for sexual maturity, this phenomenon is limited in its expression. A bird can not be sexually mature before a certain percentage of its body weight has been attained,

and the lower limit has been established in this cross at about 63 per cent (see figure 6). No significant correlation exists between the number of days to sexual maturity and maximum adult weight in the Brahmas and Leghorns or in the $F_1$ and $F_2$ hybrids. This fact would indicate that genes for sexual maturity may, to some extent, be independent of genes influencing adult body weight. Nevertheless, we must also observe the fact that early sexual maturity is in general associated with small body size; for example, the early maturing Leghorn female is small, weighing approximately 1,600 grams at adult weight, while the late maturing Brahma is large, weighing approximately 3,200 grams at adult weight. Bu the Brahma does not begin to produce eggs until it is practically full grown, and its production is low thereafter. Late maturity may therefore be, in the case of the Brahma, only one aspect of general low productive capacity. However, the $F_1$ and $F_2$ hybrid offspring resulting from a cross of these two breeds are comparatively early maturing, and the average adult body weight is intermediate, although there is an increase in the variability of adult body weight Waters (12) for the $F_2$ hybrids over both the parent races and the $F_1$ hybrids at ten wonths of age.

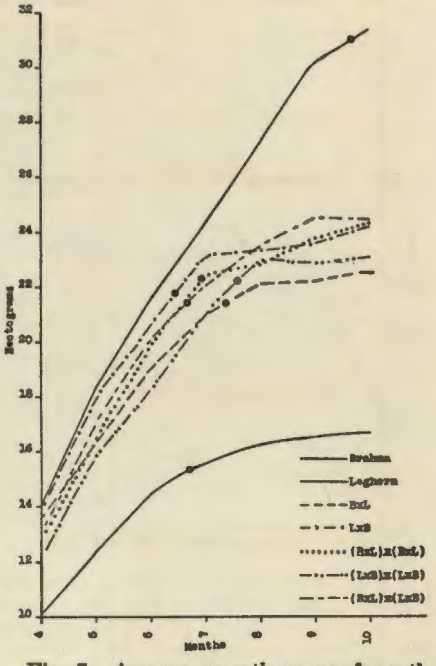


Fig. 7. Average growth curves from the fourth to the tenth month for Brahmas, Leghorns, and their $F_1$ and $F_2$ hybrid progeny. The average number of days to first egg is marked on each curve by a large dot.

The relationship of sexual maturity to growth is clearly expressed in figure 7, representing the average growth curves of the Brahma, Leghorn, and their hybrid offspring. The average number of days to first egg is marked on these curves by a large dot. The fact should be emphasized that there is a decided flattening of the growth curves after the advent of sexual maturity.

Figure 8 shows the average growth curves of $F_1$ and $F_2$ female populations separated into three groups (small, intermediate, and large) on the basis of their 10 months weight as below:

| | |
|---|---|
| Small | under 2,100 grams |
| Intermediate | between 2,100 and 2,700 grams |
| Large | over 2,700 grams |

In general, the $F_1$ and $F_2$ small, intermediate, and large individuals are early maturing like the Leghorn breed, regardless of their maximum adult weight. The growth curves for the large $F_1$ and $F_2$ individuals do not flatten after sexual maturity. Instead, the curves continue to rise for approximately three months, while the average curves previously presented

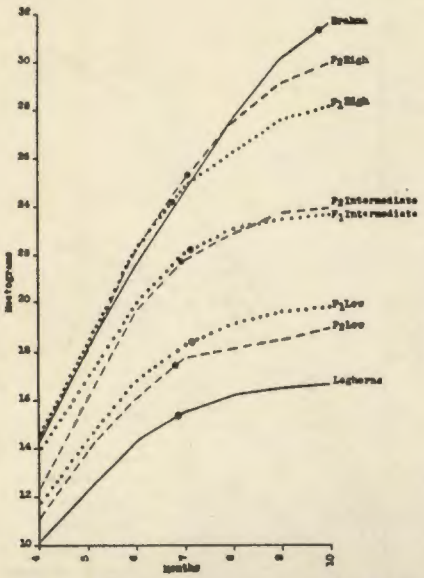

Fig. 8. Average growth curves from the fourth to tenth month of age for $F_1$ and $F_2$ females separated into three groups, each within weight limits as follows: *large,* above 2,700 grams; *intermediate,* between 2,100 and 2,700 grams; *small* below 2,100 grams. The number of individuals in each group is as follows:

| | $F_1$ | $F_2$ |
|---|---|---|
| Large | 53 | 78 |
| Intermediate | 206 | 153 |
| Small | 73 | 29 |

The average number of days to first egg is marked on each curve by a large dot.

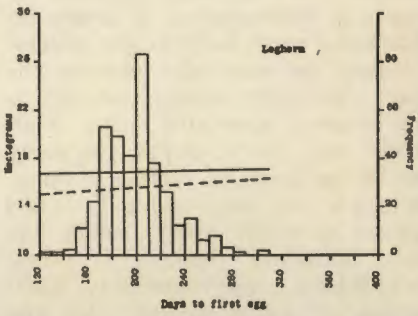

Fig. 9. Frequency polygons showing variation in age at first egg for the Leghorns, together with a broken line showing the general trend of the average body weight of these individuals at first egg and a solid line showing their ultimate average body weight.

for all groups in figure 7 show a flattening after the advent of sexual maturity.

A study of these curves leaves little doubt that genes for early maturity are, to some extent, inherited independently of genes influencing adult body size. For we find that large $F_1$ and $F_2$ individuals having an adult weight above 2,700 grams and averaging approximately 2,825 grams for the $F_1$ and 3,000 grams for the $F_2$, mature nearly as early as Leghorns which average only 1,600 grams in adult weight. Further, the $F_1$ large individuals attain an average of 86 per cent and the $F_2$ large individuals an average of 85 per cent of their adult weight at the production of the first egg. It has been demonstrated by Waters (12) that many of these large $F_2$ segregates are genetically like the Brahma in weight; nevertheless, they are early maturing like the small-sized Leghorn.

These results again emphasize the complex genetic nature of early maturity and its relationship to growth. In general, the small-sized birds mature earlier than the large-sized birds, but there are many exceptions. It has also been shown previously that there is no significant correlation between the maximum adult weight of the bird and days to first egg.

Graham (2) has demonstrated clearly with Rhode Island Reds that

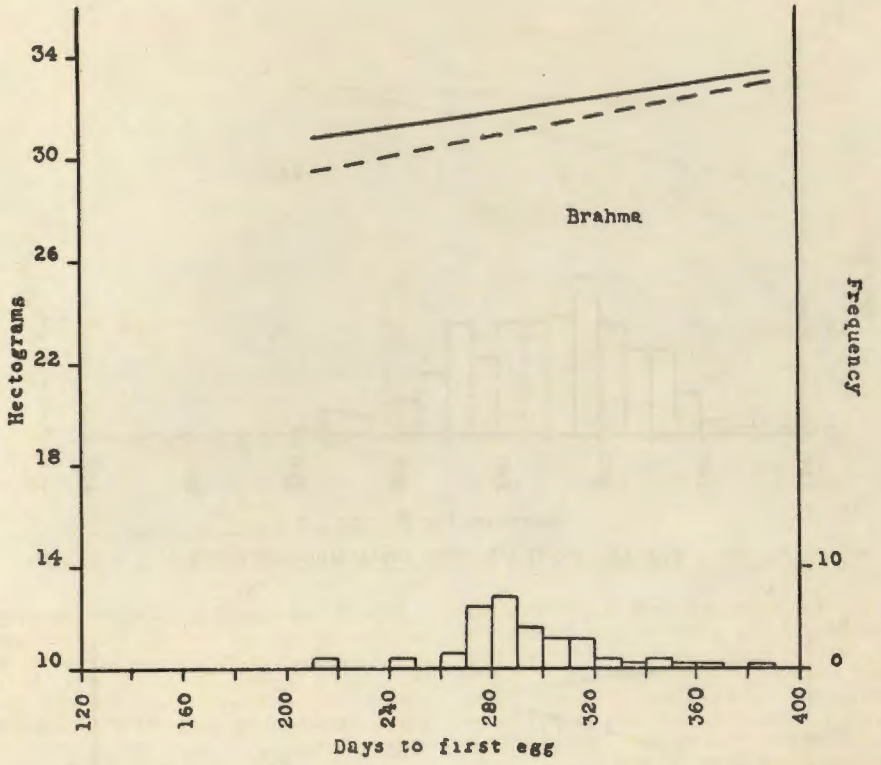


Fig. 10. Brahmas (For explanation see figure 9)

the fewer the number of days to first egg, the lower the body weight of the bird at sexual maturity. The significance of these observations should not be overemphasized, for naturally the earlier the bird commences to lay, the smaller the size of the bird, where as the late maturing bird has had a longer period in which to increase its body size. If, however, the earlier maturing bird fails to attain a maximum body weight equal to that of the late maturing bird, then an important fact has been established The material presented in figures 9-15 affords an opportunity to test this relationship.

Figure 9 shows the frequency distribution of days to first egg for the Leghorns together with a broken line showing the trend of the average body weight of these individuals at first egg and a solid line showing their ultimate average body weight. The age at first egg for the Leghorns ranges from 120 to 310 days. The broken line clearly shows that the early maturing birds are smaller at date of first egg than the late maturing birds. Graham (2) and Hays (4) demonstrate that this is also true for Rhode Island Reds. The solid line shows that the *ultimate* body weight of these early maturing birds is less than that of the late maturing birds, but this difference is not statistically significant.

Figure 10 presents the situation for the Brahma. In this case the age at first egg ranges from 210 to 390 days, with a mean of 291 days. The number of days to maximum adult weight ranges from 270 to 390 days

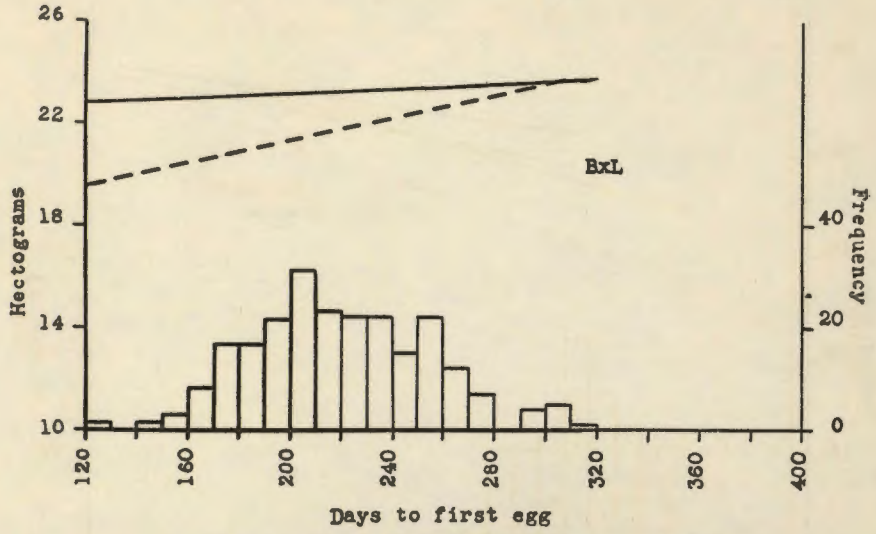


Fig. 11. $F_1$, B x L (For explanation see figure 9)

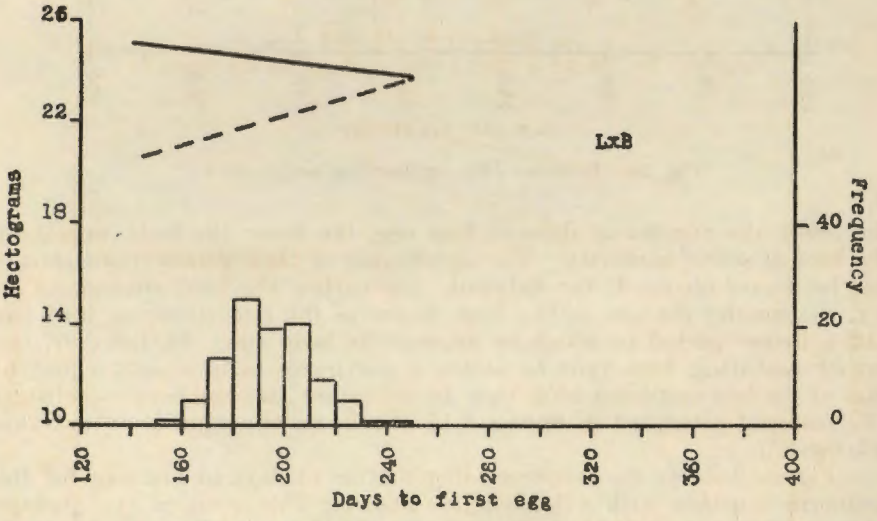


Fig. 12. $F_1$, L x B (For explanation see figure 9)

for these same birds, with a mean of 309 days. Thus we observe that, as a group, these Brahmas did not mature sexually until nearly maximum adult weight was attained. Therefore, it is to be expected that the regression line for age at first egg will be similar to the one for maximum adult weight.

Figures 11 and 12 show the frequency distribution and trend lines for the $F_1$ reciprocal hybrids. Here we find a condition similar to that found in the Leghorns. It is true that the early maturing L x B individuals

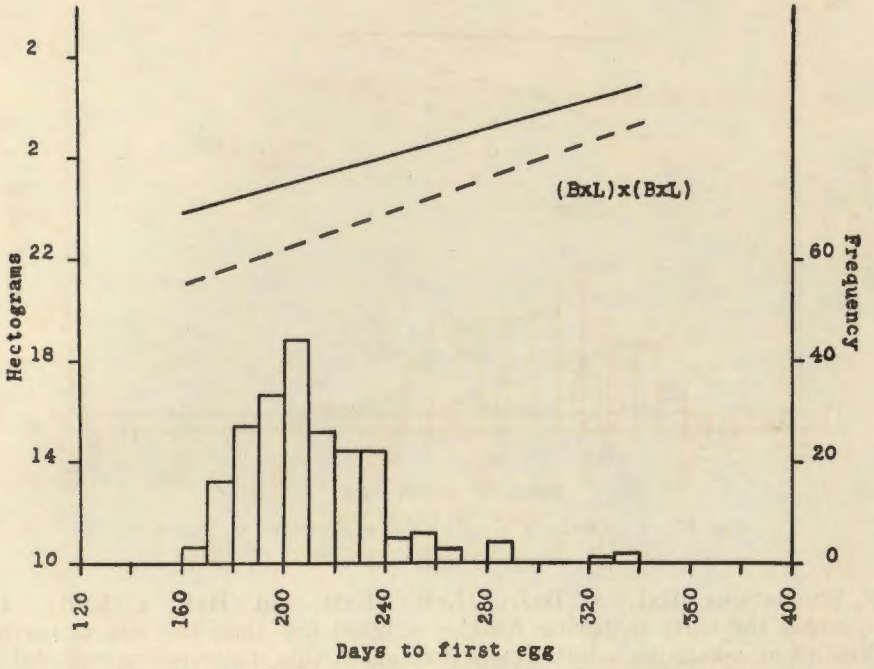


Fig. 13. $F_2$ (BxL) x (BxL) (For explanation see figure 9)

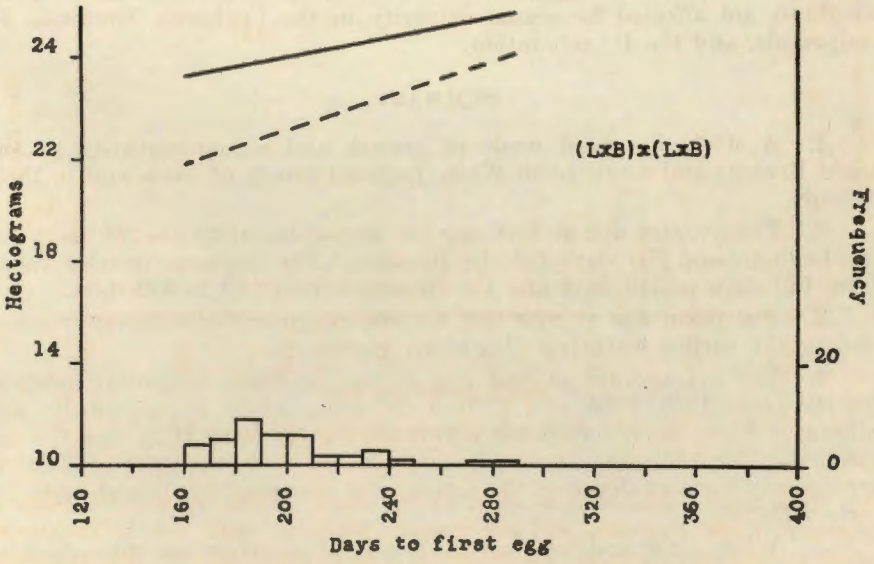


Fig. 14. $F_2$ (LxB) x LxB) (For explanation see figure 9)

do attain a slightly higher maximum body weight than the late maturing individuals but this difference is not statistically significant.

In Figures 13, 14 and 15 we find the same general situation for the

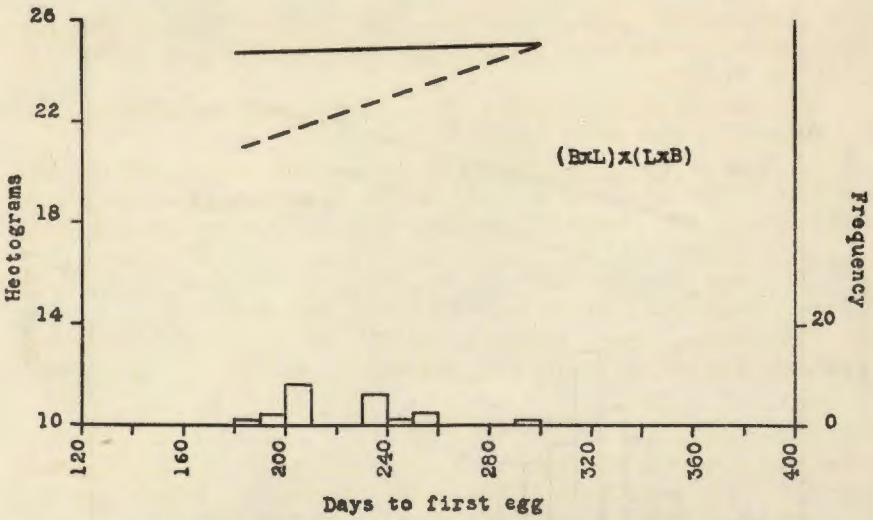


Fig. 15. $F_2$, (BxL) x (LxB) (For explanation see figure 9)

$F_2$ populations (BxL) x (BxL), (LxB) (LxB) and (BxL) x (LxB). In this case the early maturing females weighed less than the late maturing females at maximum adult weight but again this difference is not statistically significant.

It is evident, from the material presented, that the ultimate body weight is not affected by sexual maturity in the Leghorns, Brahmas, $F_1$ reciprocals, and the $F_2$ generation.

## SUMMARY

1. A study has been made of growth and sexual maturity in the Light Brahma and single comb White Leghorn breeds of fowls and in their hybrids.

2. The average age at first egg (or sexual maturity) is 200 days for the Leghorn and 291 days for the Brahma. The Leghorn females range from 120 days to 310 days and the Brahmas from 210 to 390 days.

3. The mean age at first egg for the reciprocal hybrids approaches that of the earlier maturing (Leghorn) parent.

4. The average age at first egg shows, for these reciprocal hybrids, for the years 1923, 1924, and 1926, a difference which is statistically significant. There is no significant difference for the year 1925, but the environment for 1925 was unusually favorable to early maturity. There is accordingly some evidence of the action of a dominant sex-linked gene for early maturity.

5. When early and late maturing individuals from the same flock of Leghorns are mated with the same late maturing Brahma male, we find no significant difference for age at first egg between the daughters of the two groups. This shows that the late maturing Leghorns were late for environmental reasons, not for genetic reasons.

6. For the birds used in this cross, the number of days to first egg is not a reliable criterion to use in genetically classifying individuals as early or late maturing.

7. The results obtained justify the conclusion that the inheritance of sexual maturity is of a very complex nature.

8. A study of the growth curves emphasizes the complex relationship of sexual maturity to growth and leaves little doubt that genes for sexual maturity are to some extent independent of genes influencing adult body size.

9. The correlation between the maximum adult weight of the bird and days to sexual maturity is not statistically significant.

10. The ultimate body weight is not affected by sexual maturity in the Leghorn, Brahma, $F_1$ reciprocals and the $F_2$ populations.

ACKNOWLEDGMENT

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# SWEET CORN—ITS ORIGIN AND IMPORTANCE AS AN INDIAN FOOD PLANT IN THE UNITED STATES[1]

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The fact that field corn was widely grown in North America previous to the invasion of the white man is well established. Many students of the maize plant also hold the same to be true of sweet corn. Kempton (4) states, "The common forms of maize known to us, sweet, pop, and field or horse corn, were all in existence and widely distributed when Columbus landed." In the writings of Sturtevant and others, similar statements are found. The writer has recently been afforded the opportunity of studying the archeological collections of maize in a number of the leading museums of this country and the findings of this survey lead us to call in question the above conclusions regarding sweet corn as an Indian food plant. There are two important sources of evidence bearing upon this question; namely, archeological recoveries and early literature.

## EARLY LITERATURE—SUSQUEHANNAH OR PAPOON CORN

Numerous references to the fact that sweet corn was an Indian food plant are based upon Sturtevant's (6) statement regarding the Susquehannah Indian corn, which he states was "the first sweet corn cultivated in America—and it was secured from the Indians in 1779." Sturtevant refers to this same variety in his notes (7) as Papoon corn.

The basis for his conclusion that sweet corn was an Indian food plant rests upon an article in the New England Farmer (5), written in 1822 by one who assumes the nom de plume of "Plymotheus." As to who Plymotheus was, or what his authority was for the statement regarding the identity of a plant introduced 43 years before, is not indicated. The name assumed rather suggests that he was a resident of Plymouth, Mass. A footnote by the editor refers to General Sullivan's Expedition and Bement (1) states that "sweet corn was introduced into Massachusetts in 1779 by Capt. Richard Bagnell of Plymouth." Bagnell was a member of Sullivan's Expedition, and the Journals of the Military Expedition of General Sullivan contain repeated references to fields of corn and in one it is stated that "a quantity of corn and other vegetables were destroyed." The association with the term "other vegetables" clearly signifies that the corn referred to was used for human food. The fact must be borne in mind, however, that field corn was widely used by the Indians for "roasting ears," and is so used by the white as well even to this day. The variety Early Adams, for example, which is still widely grown as a garden corn, is a type of field corn, not sweet corn (1). In fact, there is evidence to the effect that sweet corn was not prized by the Indians for green corn. Will (8), who

[1] Journal Paper No. J 145 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 293.

regards sweet corn as an Indian food plant, states, however, that the "Upper Missouri Indians rarely picked the true sweet corn green" and notes that the Papago Indians told him the same. The history of Susquehannah or Papoon corn can scarcely be accepted as other than tradition, the accuracy of which requires verification from other sources (1).

There is also evidence that sweet corn is not especially prized by the Indians as a food plant. In the early months of 1934 the writer traversed the interior of Mexico from the Rio Grande to the Yucatan border. In most of the states are to be found numerous interior villages in which the manners and customs of the people are today much as they were before the days of Cortez. In these villages one finds maize, in some form, as an important article of diet in every household. On the market seed of numerous varieties, usually based upon color distinctions, are offered. However, they were always field corn. As far as we were able to observe in the entire journey across the Republic from north to south, we were unable to secure a single specimen of sweet corn. Inquiry as to seed for "roasting or green corn" brought forth the unvarying reply, as interpreted, "This is what we use," indicating field corn. On market day, in the great Saturday market of Oxoaca, which is regarded as one of the largest typically Indian marts of the Republic, we had the same experience. Here maize, chilles and frejolies hold forth in all their glory, but not a single specimen of sweet corn was offered. Through a skilled interpreter we made repeated requests for seed for "green corn or roasting corn," and invariably field corn was offered. The numerous dialects gather here from all directions, many of them coming from distant points, representing several days' journey afoot. So far as we could learn, none of them knew of sweet corn as a plant different from field corn. Prof. C. Conzatti, author of "Flora Sinoptica Mexicana," who is widely versed in the botany of Mexico, informs me that to the best of his knowledge the aborigines (Indians) do not distinguish between field corn and sweet corn and that field corn is used for "roasting ears." Hotel keepers and vendors gave me the same information. Such a survey can be regarded as only cursory in character, but at least in the important maize regions of the Republic sweet corn is still an unknown plant.

It can scarcely be said that this plant does not exist in some of the innumerable valleys with such widely varying soil and climate. Certainly at the seat of a civilization based upon a maize culture, sweet corn mutations may even occur more frequently than elsewhere. If so, they were evidently not sufficiently prized to justify the necessary care for their propagation, and there seems warrant for the conclusion that sweet corn was not a widely cultivated nor important food plant of the aborigines of the region in which the maize plant probably originated.

The first specific reference to sweet corn in American literature which we have been able to find is contained in the letters of Timothy Dwight. During his presidency of Yale College[2] he made numerous journeys, a report of which he gave in his letters. In his second letter he notes that "maize of the kind called sweet corn is the most delicious vegetable, while in the milk stage, of any known in this country. At New Haven the sweet corn may be had in full perfection for the table by successive plantings from the middle of July to the middle of November. I commonly

[2] Now Yale University.

plant it at 12 different periods in the season." He refers to "maize as palatable, wholesome and capable of being used agreeably in more modes of cookery than any other grain." Although he was not a botanist, Dwight's notes on the native flora, and so forth, give evidence of the fact that he was a keen observer and posesessed a trained mind.

The Proceedings of the Massachusetts Horticultural Society afford important evidence regarding the origin of sweet corn. It was the custom of this society to make an annual display of fruits and vegetables, particularly of new varieties, and its yearly proceeding report in detail the varieties upon which repmiums were offered and awards made. In the society's premium list for 1838 for the first time an award is offered for "Indian corn for boiling." There were no entries and the item was dropped from the list. The list for 1846 does not include corn, but an award was made to one named Macondry for "*field corn*" and to Williams for "12 best and earliest *sweet corn.*" The fact will be noted that for the first 17 annual exhibits of the society, sweet corn is conspicuously absent. The shows were held in Boston, the most important market center of the New England states, and the fact that the first premiums offered were for "field corn for boiling" is not without significance.

The Hovey Magazine of Horticulture, established in 1835, constitutes another important source of early horticultural history. Each year the editor gave a review of "new and recently introduced vegetables worthy of general cultivation." Innumerable varieties of peas and other vegetables are passed in review, but in the first 15 years of this record not a single variety of sweet corn is listed. In 1850 Old Colony sweet corn made its appearance and to Editor Hovey it evidently tasted good as compared with "Indian corn for boiling," for he comments that "when boiled it is nothing but cream and sugar." The year 1850 marks the beginning of an important era in sweet corn development and within the next decade a number of varieties appeared, some of which, Stowell's Evergreen for example, are leading sorts to this day.

Thorburn's Seed Catalogue of 1828 lists "sugar or sweet corn" but offers no named varieties, and up to this time we find no named varieties in the literature. Sturtevant, in discussing the history of field corn, calls attention to the fact that "many varieties are always an evidence of antiquity of culture." If the converse of this proposition is equally true, then the absence of multifarious forms of sweet corn may be regarded as evidence of its youthfulness.

In brief, a review of the literature points to the conclusion that sweet corn was not a pre-Columbian food plant of the North American Indian, but rather that it is of comparatively recent introduction.

## ARCHEOLOGICAL EVIDENCE

The comment of De Candolle that "the certainty as to the origin of maize will come rather from archeological discoveries" applies with equal force to sweet corn.

Excellent archeological collections of maize are found in a number of the leading museums of the United States. The writer has been privileged to study such collections in the Field Museum, the Smithsonian Institution, the Museum of the South west and has by correspondence secured reports as to their maize collections from other leading institutions. In these collections excellent specimens of field corn are to be found representing

the dent, flint and flour types and of various colors and forms. In this survey we were quite surprised to find that sweet corn was conspicuously absent, with a single exception to which we shall refer presently. This is hardly what one would expect if sweet corn were in existence and widely distributed in pre-Columbian times, as suggested by Kempton and others.

The specimen referred to is found in the American Museum of Natural History and was collected in the Aztec ruins of New Mexico by Earl H. Morris. "These ruins," states Morris, "were built by the Chaco people during the Mesa Verde phase of Pueblo III," and he estimates that the ear in question was grown between 1200 and 1300 A.D.

The specimen was loaned the writer for determination by the courtesy of curator, Dr. Clark Wissler and is identified as *Zea mays* var. *saccharata*. The wrinkled pericarp, translucent endosperm and character of the starch grain clearly identify it as sweet corn.

The fact that this ear was recovered in the Aztec ruins of the Southwest is significant. "The evidence of archeology, history, ethnology and philology point to central and southern Mexico as the original home of maize," concludes Harshberger (3) who has made a special study of the origin and nativity of corn. If this conclusion is correct and sweet corn was a widely distributed Indian food plant, then it should be liberally represented in the archeological collections of the Southwest. Morris, however, who has made extensive archeological explorations in this region, writes[3] in reference to this Aztec ear, "I recall nothing of similar appearance among the many finds of corn that I have made in the Southwest."

## SACRED CORN

The Indians' fondness for striking colors found expression in various color patterns found in corn, such as the "sacred corn" of the Navajos, and the Zuni collection of Cushings. The Cushings collection of the Zunis contained corn of six different colors representing certain mythical figures and the seventh was supposed to represent sweet corn, but Sturtevant (6), who studied the Zuni collection, notes "there was not a sweet corn among them." Sweet varieties of corn are rather dull and lacking in color effects as compared to field corn and would appear to have but little to offer as a color appeal.

The fact may also be noted that linguistic evidence is lacking in support of the theory that sweet corn was an Indian food plant. The tribes, however widely separated, states Harshberger (3), had a common root for that important cereal (maize), thus among the Delawares we find the term "winaminge," the month of August, literally the time of roasting ears. This appelation applies equally well to field corn, a crop which is known to have been used for "roasting ears" by the Indian. So far as we have been able to learn the equivalent of the term sweet corn or sugar corn has no common root among the various tribes.

## GENETIC ASPECT

Genetic studies of the corn plant have made an important contribution to the history of its probable evolution and origin. Sturtevant classed sweet corn as a distinct species, *Zea saccharata*. The genetic evidence points to the fact it is a mutant and hence is a botanical variety of field

[3] Morris, Earl H. Letter to author under date of Nov. 20, 1933.

corn rather than a distinct species. Proof of the theory that sweet corn is a mutant of field corn is furnished by the genetic studies of Dr. E. W. Lindstrom,[4] who in 1929 discovered a single sweet corn kernel as a mutant in a series of pedigree cultures of dent corn. Four generations have been tested and crossed with normal sweet corn and all prove the original kernel to have been a true mutant from field corn. Moreover, two other similar cases of such rare mutations are recorded. In field corn there is a normal change from sugar to starch as the ear approaches maturity, whereas in sweet corn only a comparatively small number of starch grains are formed and many of these are imperfect. In other words, sweet corn is field corn in an arrested state of development and is to be regarded as younger than field corn.

Some of the varieties, such as Golden Bantam, are derived from flint corn, and others, the major portion, from the dent corns. The tendency of sweet corn, under the favorable environment of the corn belt, to revert to field-corn type of becoming more starchy or to form "starch caps" is well recognized. Sweet corn is inherently a plant of less vigor and stamina than field corn. When planted too early the seed is more likely to decay, and in the autumn it is slower in curing than the latter, and hence more subject to injury from freezing. In view of these facts, sweet corn mutations would be less likely to survive than field corn in the struggle for existence under the rugged environment of Indian agriculture.

A review of early American literature points to the conclusion that sweet corn made its appearance in the United States as a food plant near the beginning of the nineteenth century. A study of maize material in archeological collections of this country would seem to indicate that maize was comparatively rare and hence not an important Indian food plant. The single authentic archeological specimen so far recorded from the United States may be accounted for as a field corn mutant.

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# ON THE RATES OF CONTRACTION OF THE ISOLATED HEART AND MALPIGHIAN TUBE OF THE INSECT, PERIPLANETA ORIENTALIS: METHOD

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Although much experimental work has been done upon the insect heart, it has usually involved the intact animal rather than the isolated heart preparation, separated from the influences of nervous or other systems of the organism. It is well known, however, that the surviving insect heart will continue its rhythmic activity for some time when bathed with the proper fluid. Levy (1,2) employed a buffered saline solution in which the isolated hearts of the dipterous larvae, *Phormia regina* and *Calliphora erythrocephala,* remained active for four or five hours usually, but sometimes longer (up to twenty-four hours in one case). Brocher (3) used the isolated hearts of *Dytiscus marginalis* imagos and *Aeschna* larvae for qualitative observations. Koidsumi (4) used the isolated insect heart in a study of critical thermal increments but did not observe heart rate over prolonged periods. Similarly, although the rhythmic activity of the Malpighian tubes has been reported a number of times (5, 6, 7) there is lacking detailed information as to the fluctuation in activity rate when the surviving tissue is observed for prolonged periods of time.

This paper is the description of a method being used by the authors for determining the rates of contraction of the isolated heart and Malpighian tubes of the cockroach, *Periplaneta orientalis,* under controlled experimental conditions of temperature, hydrogen ion concentration, composition of immersion fluid, oxygen supply and rate of renewal of immersion fluid. The immediate objective of these experiments was to determine whether, under these conditions, the rates of heart and Malpighian tube contraction would be sufficiently "steady" to permit of their use in determining the effects of various chemical substances on insect muscle activity. The animals used were large nymphs and adults, kept in the laboratory under fairly constant conditions of room temperature and food.

## METHOD

The method of isolating the heart used here consists of first inactivating the animal by submersion for a minute or two in water or in the solution in which the dissection is to be made. The legs are severed near the body and the neck is severed as near the head as possible. The body is anchored dorsal side down to a removable wax-impregnated cardboard strip that forms part of the bottom of a rectangular, wax-covered dissecting tray, by two pins thrust through the thin lateral margins of the pronotum. With small scissors an incision is made from the anal extremity to the severed end of the neck, care being taken that the alimentary canal is not cut and that none of its contents is spilled into the hemocoele. The lateral halves of the ventral body wall are carefully separated with the points of pins and are pinned to the wax strip, inner surface uppermost, leaving the entire viscera exposed to view. The severed esophagus

is held with forceps and carefully lifted ventrally and posteriorly and, at the same time, the fat bodies are gradually cut as close as practicable to their body wall attachments. Finally, when the entire alimentary tract, with its attendant fat bodies and Malpighian tubes, is extended posteriorly from the anal end of the body wall, the rectum is pulled posteriorly until the posterior end of the heart is clearly visible; the rectum is pinned in this position, the remainder of the viscera being cut away. These operations leave the entire heart exposed to view on the inside of the dorsal body wall and, when the preparation is carefully made, vigorous contractions can be observed. If the preparation is hurriedly or carelessly made, the heart is apt to show very irregular contractions or no contractions in some or all of its parts; these effects may be due to injury of the delicate pericardium or of the wing muscles of the heart.

The Malpighian tube preparation is the same, except that the alimentary tract is left in position after lateral extension of the ventral body wall and only bits of fat body are removed to facilitate observation of the tubes.

The removable wax strip with attached heart or Malpighian tube preparation is immersed in the saline solution held by the test tube (TT) which is itself immersed in the water of the constant temperature bath held by the glass jar (J), the preparation (P) being vertical in position. Fresh saline from a Mariotte pressure bottle ($B_1$) is added to the test tube contents by drops from the dropping pipette (D) above and oxygen is supplied by bubbling air or oxygen ($A_1$) through the fluid in the test tube; the latter serves both to aerate the saline and to make it circulate, the circuit being downward on the preparation side of the wax strip (W) and upward on the other side. The saline level rises because of the added drops, until the excess is drained off through the side arm (SA) of the tube; this occurs periodically. The temperature of the saline and of the bath water is measured by the thermometers ($T_1$, $T_2$). The bath water is stirred by motor or by the bubbling through of compressed air ($A_2$) and is heated and regulated by a heater-thermostat unit (Th) that maintains temperature to about 0.2°C. The preparation is illuminated by a strong beam of light from a low voltage, incandescent lamp (L, not shown), and is observed through a binocular dissecting microscope (M, not shown) mounted horizontally upon an adjustable, rack and pinion support. Contraction rates are obtained by measuring the time required for a given number of complete contractions to occur and then converting to number of contractions per minute. In a given experiment, a single Malpighian tube is selected and its contraction rate measured throughout the entire period of observation.

This method is applicable not only to the study of isolated heart and Malpighian tubes but also to various parts of the gut. During the present observations, the hind-gut has been observed to maintain activity for as long as seven hours and the crop for almost as long. The gizzard, which does not readily continue its activity when isolated (see 8) has been noted to contract for a few minutes. The mid-gut has exhibited little or no activity.

## SALINE

The physiological saline used to bathe the preparation is one described by Levy (1) but has been slightly modified by the addition of glucose. It

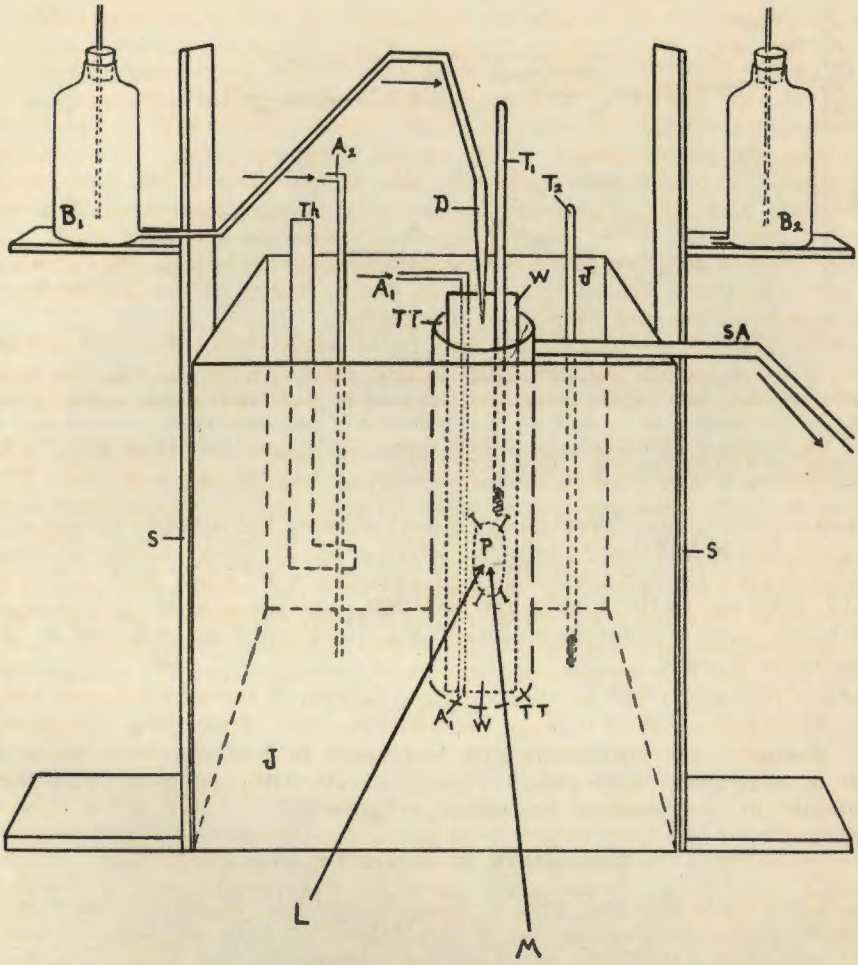


Fig. 1. Set-up used for determining rates of isolated heart and Malpighian tube contraction of the insect, *Periplaneta orientalis*, over prolonged observation periods, under conditions of constant temperature, pH, composition and rate of renewal of physiological saline. For purposes of illustration the relative size of the side arm test tube (TT) and its contents has been exaggerated and the supporting clamps for the various apparatus have been omitted.

- $A_1$, $A_2$ inlets for compressed air to physiological saline and water bath, respectively.
- $B_1$, $B_2$ bottles of saline with Mariotte-tube for maintaining constant pressure.
- D dropping pipett from which saline drops fall into test tube.
- J glass side of thermostat jar.
- L low voltage incandescent lamp used to illuminate preparation.
- M position of horizontally arranged dissecting microscope used to observe preparation.
- P heart or Malpighian tube preparation.
- SA side arm for drainage of saline from test tube.
- S, S supports for bottles of saline.
- $T_1$, $T_2$ thermometers for measuring temperature of saline and water bath, respectively.
- TT side arm test tube containing preparation, etc.
- W wax-impregnated cardboard strip that holds the preparation.

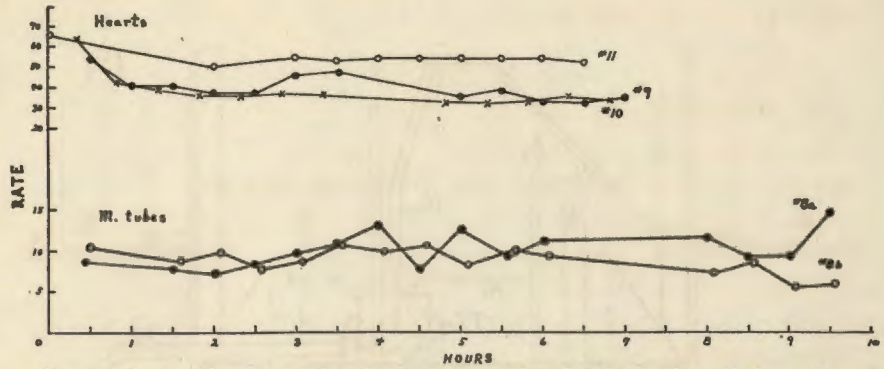


Fig. 2. Contraction rates of isolated hearts and Malpighian tubes of the cockroach, *Periplaneta orientalis*. Rates are expressed as number of contractions per minute. Hours refer to the number of hours from the beginning of the experiment. Curves No. 8a and No. 8b are at 26.5°C.; No. 9 and No. 10 at 25.0°C.; and No. 11 at 23.0°C. All are buffered to approximately pH = 7.5.

is approximately one-eleventh Levy's stock solution but contains 0.1 per cent glucose and is buffered to approximately a pH of 7.5 or 8.0 in different experiments. Its composition is 9.82 gms. NaCl, 0.77 gm. KCl, 0.50 gm. $CaCl_2$, 0.18 gm. $NaHCO_3$, 0.01 gm. $NaH_2PO_4$, 1.00 gm. glucose; this is made up to one liter of solution with distilled water and then adjusted to the required pH value.

## RESULTS

A number of experiments have been made to determine the change in rate of contraction with time. Typical results with both heart and Malpighian tube preparations are given in figure 2.

## DISCUSSION AND CONCLUSIONS

The results obtained indicate that, in general, the rate of heart beat is several times as great as that of the Malpighian tube and that the heart rate may show a relatively rapid decrease during the first hour after isolation,[1] after which it is maintained at an approximately uniform level for a period of at least six or seven hours. The Malpighian tubes have shown a surprisingly constant rate of contraction, even up to 10 hours of observation. The contraction rate of the Malpighian tube shows greater fluctuation than does heart rate but these variations are not so great as to obscure the general constancy of the Malpighian tube activity. It is apparent also that, under these experimental conditions, the rates of heart beat and Malpighian tube contraction are sufficiently "steady" over a sufficiently long time to be used in determining the effects of various dissolved substances upon insect muscle activity. In the present work it has been observed that the duration of Malpighian tube activity is increased by the presence and decreased by the absence of glucose in Levy's solution.

It is suggested that this method may be of use in determining the

[1] This accords with the report of Levy (1) that the insect hearts he used exhibited a rapid decrease in rate immediately following isolation.

direct action of certain insecticides upon the isolated insect tissue, about which little is at present known.

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# THE ALKALINE HYDROLYSIS OF CELLULOSE ACETATE RAYON

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Cellulose acetates as textiles have been hydrolyzed with alkali in the preparation of lower acetates for the spinning bath (7, 15, 21, 35, 37, 45-47, 49, 50, 57, 59, 75, 79-83, 88, 89, 93, 95, 110, 116, and 118), in the preparation of alkali cellulose (105), in the scouring (55, 56, 86, and 87), degumming (54, 74, and 101), and mercerizing (76, 77, 103, and 106) of union textiles containing cellulose acetate rayon, in the dyeing (2, 3, 5, 8, 10, 22-29, 34, 44, 52, 58, 62, 67, 71, 84, 85, 96-98, 102, 104, 107-109, 111, 117, and 119) and stripping (30 and 68) of cellulose acetate rayon, in such special finishing processes as delustering (4, 6, 16, 18, 19, 65, 66, 70, 113-115), crêping (13, 14, 32, 51, and 69), and iron-proofing (12, 17, 20, 36, 40-43, 60-62, 91, 94, 95, and 112), in processes designed to increase the resistance of a fabric of cellulose acetate rayon to wrinkling (92) or to make easier the clear cutting of pile yarns (11 and 48), and in producing decorative fabrics in which the cellulose regenerated is destroyed by carbonization (38 and 39). In most of these cases the cellulose acetate rayons have been three to twenty per cent hydrolyzed at about 70°C. in 0.025*N* to 0.3*N* alkali.

Kita, Sakurada, and Nakashima (72) have reported the fifty per cent hydrolysis of cellulose acetate by aqueous sodium hydroxide as a monomolecular reaction and they and others (1, 33, 72, 99, and 100) have used rates of hydrolysis to differentiate cellulose acetates. Haller and Ruperti (63 and 64) have described hydrolyzed cellulose acetate rayon as an unhydrolyzed center within a completely hydrolyzed surface; Colthof, Waterman, and Wolf (34) have described it as made up of intermediary layers of partially hydrolyzed ester within a completely hydrolyzed surface.

The loss of acetyl and loss of weight of a cellulose acetate rayon in fifteen minutes at 60°C. over a range of concentrations of alkali are presented in this paper.

## EXPERIMENTAL

A cellulose acetate rayon fabric of plain weave was boiled one hour at constant volume in one hundred times its weight of water, rinsed, dried, and extracted continuously for eighteen hours with anhydrous ether. The fabric prepared in this manner yielded 37.67 per cent of acetyl by Ost's (53, 90) method.

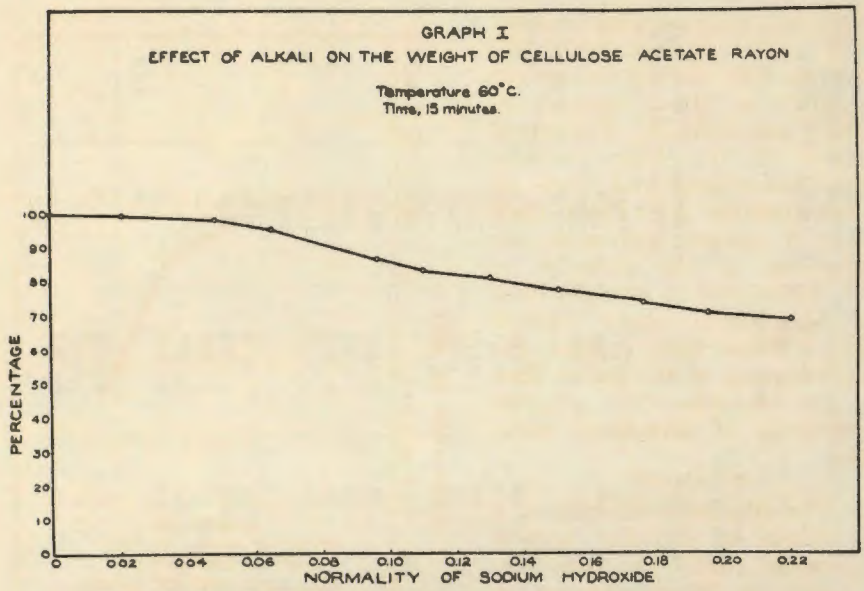
Samples of approximately one gram of the fabric were dried at 105°C. until constant and weighed with tares. Fifty cubic centimeters of a standard solution of sodium hydroxide were pipetted into 300-cc. Erlenmeyer flasks, the flasks were fitted with rubber stoppers, and placed in a water bath regulated at 60 ± 0.1°C. until the contents of the flasks attained the temperature of the bath. A sample of cellulose acetate rayon was added to each flask, the mixture was shaken during fifteen minutes at 60°C., and

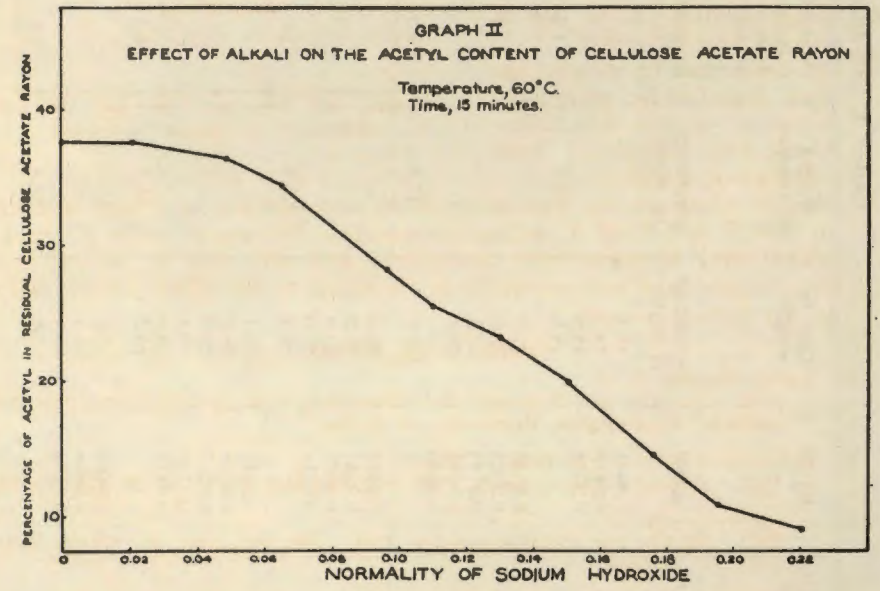
TABLE 1. *The effect of alkali on the weight and the acetyl content of cellulose acetate rayon. Temperature, 60°C. Time, 15 minutes*

| Determination | Sodium hydroxide | Rayon | Loss in weight | Weight of residue | Weight calculated from loss of acetyl | Acid required for neutralization of hydrolysate | Acetyl lost upon hydrolysis | Acetyl of residue | |
|---|---|---|---|---|---|---|---|---|---|
| number | normality | gram | percentage of rayon | percentage of rayon | percentage of rayon | cc. 0.0557 *N* | gram | percentage of rayon | percentage of residue |
| 1 | 0.0210 | 0.9365 | 0.3 | | | 18.10 | 0.0018 | 37.5 | |
| 2 | | 0.9375 | 0.1 | | | 18.10 | 0.0018 | 37.5 | |
| 3 | | 0.9624 | 0.2 | | | 18.10 | 0.0018 | 37.5 | |
| Average | | | 0.2 | 99.8 | 99.8 | | | 37.5 | 37.6 |
| 1 | 0.0489 | 0.9174 | 1.8 | | | 37.10 | 0.0163 | 35.9 | |
| 2 | | 0.9366 | 1.7 | | | 37.43 | 0.0155 | 36.0 | |
| 3 | | 0.9372 | 1.9 | | | 36.80 | 0.0170 | 35.9 | |
| 4 | | 0.9441 | 1.7 | | | 36.86 | 0.0169 | 35.8 | |
| 5 | | 0.9467 | 1.5 | | | 37.50 | 0.0153 | 36.0 | |
| Average | | | 1.7 | 98.3 | 98.3 | | | 35.9 | 36.5 |
| 1 | 0.0652 | 0.9522 | 4.6 | | | 39.92 | 0.0446 | 33.0 | |
| 2 | | 0.9539 | 4.7 | | | 39.23 | 0.0462 | 32.8 | |
| 3 | | 0.9512 | 4.7 | | | 39.90 | 0.0446 | 33.0 | |
| 4 | | 0.9506 | 4.9 | | | 38.76 | 0.0474 | 32.7 | |
| Average | | | 4.7 | 95.3 | 95.3 | | | 32.9 | 34.5 |
| 1 | 0.0965 | 0.9122 | 13.0 | | | 36.50 | 0.1201 | 24.5 | |
| 2 | | 0.9342 | 12.8 | | | 36.35 | 0.1205 | 24.8 | |
| 3 | | 0.9307 | 13.3 | | | 35.10 | 0.1235 | 24.4 | |
| Average | | | 13.0 | 87.0 | 87.2 | | | 24.6 | 28.2 |
| 1 | 0.1100 | 0.9397 | 17.0 | | | 32.50 | 0.1587 | 20.8 | |
| 2 | | 0.9209 | 15.7 | | | 38.12 | 0.1453 | 21.9 | |
| 3 | | 0.9694 | 16.3 | | | 32.35 | 0.1591 | 21.3 | |
| 4 | | 0.9416 | 15.8 | | | 36.12 | 0.1501 | 21.7 | |
| Average | | | 16.2 | 83.8 | 84.0 | | | 21.4 | 25.6 |

TABLE 1. (Continued)

| Determination number | Sodium hydroxide normality | Rayon gram | Loss in weight percentage of rayon | Weight of residue percentage of rayon | Weight calculated from loss of acetyl percentage of rayon | Acid required for neutralization of hydrolysate cc. 0.0557 *N* | Acetyl lost upon hydrolysis gram | Acetyl of residue percentage of rayon | Acetyl of residue percentage of residue |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.1300 | 0.9341 | 18.4 | | | 44.05 | 0.1741 | 19.0 | |
| 2 | | 0.9413 | 18.5 | | | 43.03 | 0.1765 | 18.9 | |
| 3 | | 0.9508 | 18.6 | | | 41.35 | 0.1806 | 18.7 | |
| Average | | | 18.5 | 81.5 | 81.6 | | | 18.9 | 23.2 |
| 1 | 0.1502 | 0.9528 | 22.3 | | | 45.26 | 0.2146 | 15.1 | |
| 2 | | 0.9525 | 23.5 | | | 40.50 | 0.2261 | 13.9 | |
| 3 | | 0.9784 | 22.1 | | | 43.90 | 0.2179 | 15.4 | |
| 4 | | 0.9045 | 20.8 | | | 55.23 | 0.1908 | 16.6 | |
| 5 | | 0.9860 | | | | 46.93 | 0.2106 | 16.3 | |
| Average | | | 22.2 | 77.8 | 78.3 | | | 15.5 | 19.9 |
| 1 | 0.1760 | 0.9076 | 26.5 | | | 54.98 | 0.2469 | 10.5 | |
| 2 | | 0.9581 | 26.2 | | | 50.98 | 0.2564 | 10.9 | |
| 3 | | 0.9067 | 25.9 | | | 57.58 | 0.2406 | 11.1 | |
| 4 | | 0.9589 | 27.0 | | | 47.10 | 0.2657 | 10.0 | |
| Average | | | 26.4 | 73.6 | 73.6 | | | 10.6 | 14.4 |
| 1 | 0.1957 | 0.9312 | 30.4 | | | 56.28 | 0.2861 | 7.0 | |
| 2 | | 0.9508 | 28.6 | | | 60.35 | 0.2764 | 8.6 | |
| 3 | | 0.9557 | 29.2 | | | 58.15 | 0.2816 | 8.2 | |
| 4 | | 0.9160 | 30.4 | | | 57.13 | 0.2841 | 6.7 | |
| 5 | | 0.9474 | 29.9 | | | 56.40 | 0.2858 | 7.5 | |
| Average | | | 29.7 | 70.3 | 70.6 | | | 7.6 | 10.8 |
| 1 | 0.2202 | 0.9488 | 32.5 | | | 68.13 | 0.3104 | 5.0 | |
| 2 | | 0.9628 | 31.1 | | | 71.12 | 0.3033 | 6.2 | |
| 3 | | 0.9708 | 30.1 | | | 74.95 | 0.2941 | 7.4 | |
| Average | | | 31.2 | 68.8 | 69.2 | | | 6.2 | 9.0 |



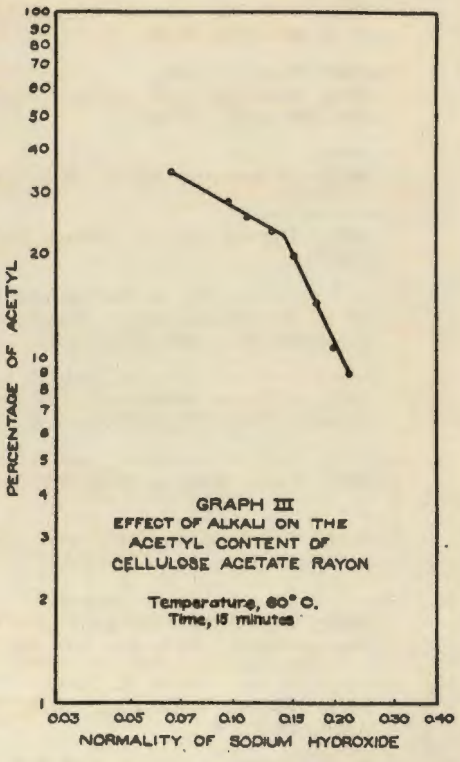




then placed in an iced bath for titration with 0.0557 *N* hydrochloric acid in the presence of phenolphthaléin. The residual fabric was removed after titration of the hydrolysate, rinsed three times for twenty minutes each by shaking with 200 cc. of water, and dried to constant weight.

The data have been selected from five or more determinations at each concentration of alkali and are presented in table 1 and in graphs I, II, and III.

Acetyl values for the residual fiber corresponding to cellulose diacetate,

34.96, and cellulose monoacetate, 21.08, were obtained upon hydrolysis with sodium hydroxide at 60°C. in fifteen minutes at 0.06 *N* and 0.142 *N*. The acetyl values of the fiber between these normalities and those at concentrations greater than 0.142 *N* sodium hydroxide are functions, of the form $y = ax^b$, which plot as straight lines on logarithmic paper (graph III). These data suggest the participation of the entire fiber in the stepwise (78) alkaline hydrolysis of acetylated cellulose.




GRAPH III
EFFECT OF ALKALI ON THE ACETYL CONTENT OF CELLULOSE ACETATE RAYON
Temperature, 60° C.
Time, 15 minutes

## SUMMARY

1. The alkaline hydrolysis of a fabric of cellulose acetate rayon (37.67 per cent acetyl) in fifteen minutes at 60°C. has been followed by determination of the loss in weight and in acetyl.
2. The decrease in weight has been found to be, within the accuracy of the determinations, that calculated from the loss of acetyl.
3. Acetyl values for the diacetate and the monoacetate have been obtained at 0.06 *N* and 0.142 *N* sodium hydroxide. The acetyl value of the residual fiber between these normalities and also that at greater concentrations have been shown to be functions of the alkali concentration of the form $y = ax^b$.

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# THE DECOMPOSITION OF STRAW IN THE PRODUCTION OF ARTIFICIAL MANURE[1]

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Many different kinds of organisms are active in the decomposition of plant materials and many intermediate products are formed which are subject to further decomposition. Under certain conditions, however, the decomposition of plant materials may lead to the formation of more or less stable products resistant to further decomposition under those conditions. Certain constituents are readily attacked by a large variety of microorganisms, whereas, other constituents are subject to attack by a relatively small number of microorganisms. The latter constituents are said to be more resistant to decay and under certain conditions they may tend to accumulate. Lignin is a plant constituent not as readily attacked by the common soil microorganisms as some other plant constituents and hence it tends to accumulate in the soil.

In a series of experiments on the production of artificial manure (4) results were obtained which indicated that lignin decomposed rather readily or was altered in such a way that it became soluble in strong acids. The study has been continued and the present paper is a report of the results obtained.

## EXPERIMENTAL

### MATERIALS AND METHODS

In an experiment on the production of artificial farm manure from oat straw, four ricks, each containing 1,000 pounds of dry oat straw were treated on August 1, according to the outline given in table 1.

TABLE 1. *Outline of treatments*

| Compost No. | Treatment |
|---|---|
| 1 | 1,000 lbs. oat straw + Adco |
| 2 | 1,000 lbs. oat straw + cyanamid and rock phosphate* |
| 3 | 1,000 lbs. oat straw + ammonium sulfate, superphosphate and limestone** |
| 4 | 1,000 lbs. oat straw |

*37.5 lbs cyanamid and 37.5 lbs. 200-mesh rock phosphate
**75 lbs. mixture containing:
45 percent ammonium sulfate
15 per cent superphosphate (16 per cent)
40 per cent 100-mesh limestone

Water was added at the rate of 250 gallons per ton of dry straw when the composts were made and no more water was added except that which fell as rain or snow.

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The composts were sampled three times the following year and the composts were thrown out of the ricks, reworked completely, then packed back into the ricks after each sampling. The samples were taken on April 2, July 7 and October 16. A sharp spade was used to cut a sample about a foot square from the top to the bottom of the compost. The sample was taken each time near the center of the compost. The samples were air-dried and ground to pass a 70-mesh screen. Nitrogen was determined in all samples by the Kjeldahl-Gunning method. One-gram samples were ignited in an electric muffle furnace at 800°C. for ash determinations. The amount of organic matter soluble in 6 per cent hydrogen peroxide was determined by the method of Robinson and Jones (3). The degree of decomposition of each manure at each sampling was calculated.

TABLE 2. *Analyses of straw and of composts sampled April 2*

| Compost No. | Percentage | | | |
|---|---|---|---|---|
| | Nitrogen | Soluble in 6% $H_2O_2$ | Ash | Decomposed |
| Straw* | 0.71 | 15.75 | 8.55 | — — |
| 1 | 2.74 | 13.91 | 36.81 | 23.18 |
| 2 | 1.97 | 13.72 | 36.75 | 22.81 |
| 3 | 2.71 | 11.43 | 27.36 | 16.32 |
| 4 | 2.16 | 12.13 | 18.18 | 15.17 |

* Sample of straw used in compost.

In addition to the above determinations, the organic carbon, pentosan,[2] cellulose, lignin, hot water-soluble and ether-soluble materials were determined on the samples taken on July 7 and October 16.

The pentosan content was determined by the A.O.A.C. method. Cellulose was determined by the method of Mehta (1). The 72 per cent sulfuric acid method for lignin as modified by Ritter, Seborg and Mitchell (2) was followed. The hot water-soluble material was determined by boiling a one-gram sample in 100 cc. of distilled water 30 minutes under a reflux and determining the solids in an aliquot taken for evaporation. The ether extract was determined similarly in a Bailey-Walker fat extraction apparatus. The organic carbon was determined by the dry combustion method.

## RESULTS

Some decomposition had taken place in all composts during the period from August to the following April as is evidenced in the analyses shown in table 2. A considerable increase in the nitrogen content of all manures occurred. The increases in the percentage of ash in all composts indicate considerable decomposition. A superficial examination of the composts indicated a degree of decomposition approximately the same as shown by the data in the table.

The data in table 3 show that the percentage of nitrogen in all composts was lower at the July 7 sampling than at the April 2 sampling. The pentosan content was decreased considerably in all composts below the amount contained in the original straw. The cellulose content of all com-

[2] Furfuraldehyde-yielding constituents.

TABLE 3. *Analyses of straw and of composts sampled July 7*

| Compost No. | Percentage | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ether extract | Hot water extract | Soluble in 6% $H_2O_2$ | Pento-san | Cellu-lose | Lignin | Ash | Decom-posed | Organic carbon | Nitro-gen |
| Straw | 1.15 | 14.07 | 15.75 | 20.99 | 26.11 | 19.00 | 8.55 | —— | 41.81 | 0.71 |
| 1 | 0.57 | 10.62 | 21.22 | 6.61 | 9.54 | 22.78 | 46.34 | 39.54 | 29.49 | 2.36 |
| 2 | 0.22 | 7.66 | 23.32 | 8.91 | 11.74 | 25.16 | 52.25 | 48.83 | 29.75 | 1.86 |
| 3 | 0.36 | 10.49 | 21.92 | 10.17 | 12.26 | 32.41 | 32.31 | 32.47 | 35.86 | 2.23 |
| 4 | 0.43 | 13.41 | 28.58 | 13.40 | 17.50 | 30.74 | 9.60 | 33.61 | 42.89 | 1.49 |

TABLE 4. *Analyses of straw and of composts sampled October 16*

| Compost No. | Percentage | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ether extract | Hot water extract | Soluble in 6% $H_2O_2$ | Pento-san | Cellu-lose | Lignin | Ash | Decom-posed | Organic carbon | Nitro-gen |
| Straw | 1.15 | 14.07 | 15.75 | 20.99 | 26.11 | 19.00 | 8.55 | —— | 41.81 | 0.71 |
| 1 | 0.26 | 9.02 | 27.91 | 3.72 | 8.33 | 23.79 | 56.43 | 64.08 | 23.58 | 1.56 |
| 2 | 0.21 | 7.30 | 23.83 | 3.20 | 6.07 | 19.89 | 63.64 | 65.62 | 18.91 | 1.36 |
| 3 | 0.56 | 13.14 | 24.90 | 8.86 | 11.44 | 27.51 | 34.40 | 37.93 | 32.59 | 2.21 |
| 4 | 0.39 | 13.10 | 21.78 | 7.17 | 10.89 | 31.53 | 41.03 | 36.97 | 22.19 | 1.72 |

posts was also decreased to a large extent and the disappearance of cellulose paralleled somewhat the loss of pentosans but to a slightly less extent. The percentage of lignin increased slightly in composts 1 and 2 and to a much greater extent in composts 3 and 4 over that found in the original straw. There was a marked increase in the percentage of ash in all composts except 4. The amount of materials soluble in hot water decreased in all composts but to a much greater extent in compost 2. The percentage of ether-soluble substances was highest in the original straw and lowest in compost 2. The percentage of material soluble in hydrogen peroxide was increased at the July 7 sampling over that of the April 2 sampling. The degree of decomposition as calculated from these data indicates the largest decomposition of straw in compost 2 and the least decomposition in compost 3.

The analyses of the composts at the October 16 sampling are given in table 4.

The nitrogen content of composts 1, 2 and 3 decreased slightly, whereas, it increased in compost 4. The pentosan content was reduced to a small percentage in all composts. The percentage of cellulose in all composts was considerably decreased over that present at the July sampling. The percentage of lignin, though still higher than that of the original straw, was decreased considerably in composts 2 and 3 over that present at the July 7 sampling. The ash content of all manures increased materially during the period from July 7 to October 16. The degree of decomposition was high in composts 1 and 2 and relatively low in composts 3 and 4.

TABLE 5. *Analyses of manures on the basis of original organic matter*

| Compost No. | Percentage | | | | | |
|---|---|---|---|---|---|---|
| | Sampled July 7 | | | Sampled October 16 | | |
| | Pentosan | Cellulose | Lignin | Pentosan | Cellulose | Lignin |
| Straw | 20.99 | 26.11 | 19.00 | 20.99 | 26.11 | 19.00 |
| 1 | 1.22 | 1.76 | 5.11 | 0.43 | 0.97 | 2.75 |
| 2 | 1.46 | 1.92 | 4.15 | 0.43 | 0.81 | 2.71 |
| 3 | 2.68 | 3.24 | 8.57 | 2.15 | 2.84 | 6.92 |
| 4 | 10.98 | 14.34 | 25.13 | 1.50 | 2.27 | 6.58 |

The data show a decrease in easily decomposable constituents of the straw and an increase in the ash and $H_2O_2$-soluble content of the composts as decomposition proceeds. The degree of decomposition correlates fairly well with the disappearance of certain constituents of the straw, such as organic carbon, pentosans and cellulose. Compost 2 was 65.62 per cent decomposed at the October 16 sampling and the lignin content was 19.89 per cent, an increase of 0.89 per cent lignin. These data show that lignin decomposes more slowly than pentosans and cellulose but that the lignin has undergone some decomposition. If the percentage of lignin in the manures could be calculated upon the basis of the original organic matter or straw, the extent of decomposition of the lignin would be apparent. An estimate of the lignin content of the manures based upon the original organic matter by means of the ash and loss on ignition data was made. The results are given in table 5.

The pentosan content of the composts 1, 2 and 3 was reduced to a small percentage at the July 7 sampling, whereas, the untreated straw still contained a large percentage of this constituent. At the October 16 sam-

pling the pentosans had been reduced to a minimum in all composts. The cellulose content of all composts had largely disappeared at the October 16 sampling. The percentage of lignin calculated on the basis of original organic matter was low in all composts at both samplings, except in the case of compost 4 at the July 7 sampling. The low lignin content of all manures at the October 16 sampling shows that lignin has disappeared and at a rather surprisingly rapid rate compared with the disappearance of pentosans and cellulose.

## SUMMARY AND CONCLUSIONS

Samples of composted straw were taken at intervals over a period of 15 months. Analyses of the composts at the end of the period showed that the cellulosic and furfuraldehyde-yielding constituents had largely disappeared and that 60 to 90 per cent of the lignin was decomposed. The loss in organic carbon was parallel to the gain in ash constituents. The material soluble in 6 per cent hydrogen peroxide increased slightly and that soluble in hot water and in ether decreased slightly as decomposition proceeded. With favorable environmental conditions these constituents would no doubt have decomposed more rapidly than they did in this experiment.

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# THE BUTYL-ACETONIC FERMENTATION IN SUGAR MEDIA[1]

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Donker (1926) and Van der Lek (1930) have studied extensively the butyl-acetone fermentation and have reported good production of 'solvents' from glucose in the presence of either yeast or peptone as the source of nitrogen. In general, the fermentations were more nearly complete with yeast extract.

Robinson (1922) divided the mono-saccharides into two groups on the basis of their dissimilation by *Clostridium acetobutylicum*. The one group, including glucose, fructose and mannose, gave the characteristic acid curve of the butyl-acetonic fermentation, the acidity rising rapidly to a maximum followed by a sharp decline. In the second group of sugars including xylose and galactose, there was little drop in the acidity following the maximum.

Peterson, Fred and Schmidt (1924) reported normal yields of 'solvents' from xylose, arabinose and glucose in a semi-synthetic medium, while Wilson and Fred (1929) reported good yields of 'solvents' from starch with beef peptone, beef aminoids, casein or casein aminoids as the source of nitrogen and obtained curves showing the typical drop in acidity.

Wynne (1931) studying the effect of acids upon the butyl-acetone fermentation, concluded that in general, complete inhibition of fermentation occurred within a pH zone of 3.9 to 3.65.

Weinstein and Rettger (1932) were unable to corroborate the observations of Robinson and found that in Robinson's medium with xylose, glucose, starch and sucrose the acidity curves produced by their organisms showed little drop following the maximum acidity. Under these conditions, the fermentations gave rise to normal yields of acetone with little or no butyl alcohol. After further investigations they concluded that a prolamine or alcohol-soluble protein is necessary in order that glucose may be fermented in a semi-synthetic medium by *Cl. acetobutylicum* and normal yields of acetone and butyl alcohol be formed.

In initiating the experiments herein reported, it became evident that the organisms used were capable of consistently producing good yields of 'solvents' from glucose in Robinson's semi-synthetic medium with only peptone as a source of nitrogen. The cultures used throughout much of the work were received from Rettger and used by Weinstein and Rettger (1932) in their investigation. It became apparent that our results were not in agreement with those of Weinstein and Rettger. It was felt desirable therefore to determine the extent to which the yields of 'solvents' produced by these organisms agree with the yields reported by other investigators and to discover, if possible, some explanation for divergent results.

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## METHODS

The stock medium used throughout these experiments consisted of five per cent corn mash, prepared by adding one liter of water to 50 gms. of yellow corn meal and autoclaving at 20 pounds pressure for one hour on two successive days. The loss due to evaporation was made up with sterile water and the medium distributed aseptically in sterile tubes and heated for one hour in flowing steam. The tubed medium was then incubated for four days at 37°C. before using.

Glucose was sterilized separately in aqueous solution and added to the medium at the time of inoculation.

The inorganic medium, unless otherwise noted, was that suggested by Robinson and had the following composition:

| | |
|---|---|
| Potassium di-hydrogen phosphate | 1.00 gm. |
| Magnesium sulphate | 0.20 gm. |
| Sodium chloride | 0.01 gm. |
| Ferrous sulfate | 0.01 gm. |
| Distilled water | 1,000.00 ml. |

To this basal medium, were added three per cent of glucose and the source of nitrogen.

### PREPARATION OF INOCULUM

The sporulated cultures, activated by pasteurizing in boiling water for 45 seconds, were inoculated into a tube of corn mash and incubated at 37°C. After vigorous fermentation had set in, one ml. transfers were made at daily intervals to fresh corn-mash tubes. After three days 10 ml. of a 24 hour corn-mash culture (unless otherwise specified) were used to start the large fermentations. Strips of filter paper were added to fermentations in liquid media, to assist in the development of anaerobic conditions. All cultures were allowed to sporulate at frequent intervals during the course of the investigation and then pasteurized, a procedure found necessary in order to maintain high solvents production.

Cultures of *Cl. acetobutylicum* used in this investigation are as follows: B, I, D, R and So received from Dr. Leo Rettger; 12A and 12B are Fernbach cultures furnished by Dr. L. M. Christensen and 14c was received from Dr. A. M. Wynne. Our appreciation is expressed to the donors.

Cultures were examined for purity at frequent intervals, and chemical analysis of the fermentation products gave added assurance that we were dealing with typical *Cl. acetobutylicum*.

Systematic study of these strains along with closely related forms has been made in this laboratory and will appear as a separate contribution. The results show that the characters of the strains used had not significantly changed from those originally described by the authors.

### ANALYTICAL METHODS

Acetone was determined on aliquots of the distillate obtained as described later, by Goodwin's (1920) modification of Messinger's method.

Alcohols were determined by two methods. The first method, developed by Weyer and Rettger (1927), consists in direct distillation of an aliquot of the fermentation liquor into a cooled Babcock butter-fat bottle which is filled to the shoulder with anhydrous potassium carbonate. The 'solvents' are salted out on standing and accumulate as an oily layer

in the neck of the bottle. The volume is read in milliliters. The amount of acetone in grams, previously determined, divided by 0.7962 (specific gravity of acetone) gives the total yield of acetone in milliliters. This value, subtracted from the total volume of 'solvents,' gives the volume in milliliters of alcohol, which, when multiplied by 0.8057, (specific gravity of butyl alcohol) is converted into grams. The method assumes that the only solvents produced are acetone and butyl alcohol and that there is no volume contraction following their mixture. It provides, however, a rapid, reasonably accurate and, at least, comparable method for evaluating the 'solvents' production by the butyl organism.

In the second method, an aliquot is distilled using an iced condenser. The distillate is made alkaline to phenolphthalein and redistilled, the second distillate is brought accurately to a volume of 200 ml. with distilled $CO_2$-free water. Aliquots of the distillate are used for the determination of acetone as previously mentioned.

For the determination of acetone and ethyl alcohols in the distillate, an unpublished method developed by Stahly, Osburn and Werkman based on oxidation of the alcohols and determination of the acids in the resulting solution by partition with ethyl ether, was used. The method consists in adding 50 ml. of the distillate to 10 gm. of potassium dichromate and 25 ml. of ortho-phosphoric acid (85 per cent) contained in a 200 ml. balloon flask. The flask is connected to an efficient reflux condenser and heated at such a rate that the mixture is brought to a boil in two minutes. Gentle boiling is continued for six minutes and the flask cooled and transferred, (after washing down the reflux condenser) to a Leibig condenser. Distillation is continued until the residue foams and nearly fills the flask. The distillate is made up to a volume of 200 ml. and aliquots of the acid solution are partitioned with ethyl ether. From the constants so determined, the percentages of butyric and acetic acids in the acid distillate are determined and hence the quantities of butyl and ethyl alcohols in the neutral distillate.

In all cases, 'solvents' are reported in percentage by weight of the substrate added.

Titrable acidity was determined by diluting 10 ml. aliquots of the liquor to 50 ml. with $CO_2$-free distilled water, heating to boiling and titrating with 0.05 normal NaOH in the presence of phenolphthalein.

Oxidation-reduction potentials were determined by the use of the electron tube potentiometer as described by Werkman, Johnson and Coile (1933).

Determinations of the pH were made with the electron tube potentiometer and quinhydrone electrode.

Zein was prepared in the following manner. Corn gluten is extracted continuously for 48 hours with ethyl ether to remove the corn oils and other fatty constituents. The residue is extracted for an additional 48 hours in a stream of water and after drying, provided the base for the extraction of the zein. The latter is accomplished with two to three extractions by decantation, using 85 per cent ethyl alcohol and allowing the gluten to remain in contact with the alcohol for 48 hour periods. The alcohol is partly removed from the resulting solution by distillation at 50 to 55°C. under reduced pressure. The zein precipitates from the resulting syrupy solution by pouring it slowly into ice cooled water containing a small amount of salt, and is purified by resolution in alcohol followed by a second precipitation. The product gives positive biuret and xanthoproteic reactions.

## EXPERIMENTAL

The first series of fermentations was set up as an orientating experiment, and was carried out in 500 ml. cotton stoppered flasks containing 400 ml. of medium. Each flask was inoculated with 10 ml. of a 24 hour corn-mash culture of strain I. Analyses were made after 96 hours. The results of this experiment are shown in table 1.

TABLE 1. *Production of 'solvents' from glucose in Robinson's medium with various nitrogen sources*

| No. | Robinson's medium with 3 per cent glucose plus | Alcohol by salting out | Acetone | Alcohols by oxidation | |
|---|---|---|---|---|---|
| | | | | EtOH | BuOH |
| 1 | | —— | 6.34 | 5.03 | 17.59 |
| 2 | Aqueous extract of 20 gm. corn gluten | 15.58 | 5.83 | 1.30 | 17.87 |
| 3 | Aqueous extract of 20 gm. gluten + 20 gm. pulped filter paper | 13.70 | 6.40 | 1.17 | 15.21 |
| 4 | 5 per cent yeast | —— | 4.51 | 1.58 | 15.53 |
| 5 | 5 per cent corn zein | —— | 6.80 | 1.02 | 14.80 |
| 6 | 1 per cent corn zein | —— | 5.19 | 0.22 | 13.78 |
| 7 | 5 per cent corn mash only | 17.98 | 7.02 | 1.20 | 19.49 |

The data in table 1 show no significant difference in the yields of 'solvents.' In view of these results, unlike those obtained by Weinstein and Rettger (1932), it seemed desirable to follow the course of the acidity produced in glucose medium. One-liter flasks each containing 800 ml. of Robinson's inorganic medium with 0.5 per cent Bacto peptone and 3 per cent of glucose (C.P.), were inoculated with 10 ml. of a 24 hour corn-mash culture of the various strains of *Cl. acetobutylicum*. The flasks were so arranged that samples could be withdrawn at intervals and the acidity determined.

The results presented in table 2 and in part in figure 1 show reasonably sharp breaks in the acidity curves in all cases, with the possible exception of strain So. This is in good agreement with the results of Robinson (1922) but fails to confirm those of Weinstein and Rettger who found little decline in the acidity curve following its maximum when the organisms were grown in the mineral medium with peptone as the only source of nitrogen.

Four of the previously used cultures, B, I, K and St were then inoculated into flasks containing 400 ml. of Robinson's medium with 3 per cent glucose. A 5 per cent corn mash control was run and 'solvents' determined after four days' incubation at 37°C. The results are shown in table 3.

In all of these fermentations good yields of 'solvents' were obtained from glucose in Robinson's medium. It will be noted that in all cases the yields of ethyl alcohol in the glucose medium were markedly higher than in the corn mash. In a report by Wilson and Fred (1929) it was observed that when starch is fermented in a medium containing only peptone as a source of nitrogen, there is a slight rise in the acetone at the expense of ethyl alcohol.

TABLE 2. *Rate of acid production in Robinson's medium**

| Strain | B | I | K | R | So | St | 12A | 12B |
|---|---|---|---|---|---|---|---|---|
| Time in hrs. | | | | | | | | |
| 0 | 3.00 | 3.00 | 3.00 | 3.25 | 3.60 | 2.95 | 3.20 | 3.25 |
| 14 | 8.05 | 7.40 | 8.10 | 8.20 | 8.85 | 6.40 | 3.30 | 3.30 |
| 19.5 | 8.80 | 9.10 | 9.00 | 8.90 | 11.20 | 9.00 | 3.40 | 3.30 |
| 23.5 | 8.60 | 8.70 | 8.20 | 8.40 | 11.70 | 9.10 | 3.65 | 3.30 |
| 27.5 | 7.55 | 8.20 | 6.75 | 6.30 | 11.90 | 9.10 | 3.90 | 3.60 |
| 31.5 | 7.30 | 7.00 | 6.40 | 6.90 | 12.00 | 9.50 | 5.50 | 3.70 |
| 40.0 | 6.10 | 6.00 | 6.00 | 6.10 | 12.00 | 6.60 | 9.20 | 6.25 |
| 46 | 6.00 | 6.20 | 6.00 | 6.60 | 11.50 | 6.30 | 10.50 | 8.90 |
| 52 | 5.90 | 6.40 | 6.00 | 6.80 | 11.30 | 6.60 | 10.20 | 11.10 |
| 56.5 | 6.20 | 7.00 | 6.30 | 7.10 | 11.30 | 6.50 | 9.40 | 11.50 |
| 65.5 | 6.60 | 6.60 | 6.30 | 6.70 | 11.10 | 6.70 | 8.00 | 10.20 |
| 71.5 | 6.50 | 6.30 | 6.20 | 6.70 | 10.50 | 6.90 | 7.60 | 9.50 |
| 79 | 6.10 | 6.40 | 6.10 | 6.70 | 10.40 | 6.70 | 8.50 | 9.10 |
| 91.5 | 6.30 | 6.20 | 6.00 | 6.70 | 10.40 | 5.90 | 8.50 | 8.70 |

* Cc. 0.1 N KOH per 10 cc. culture.

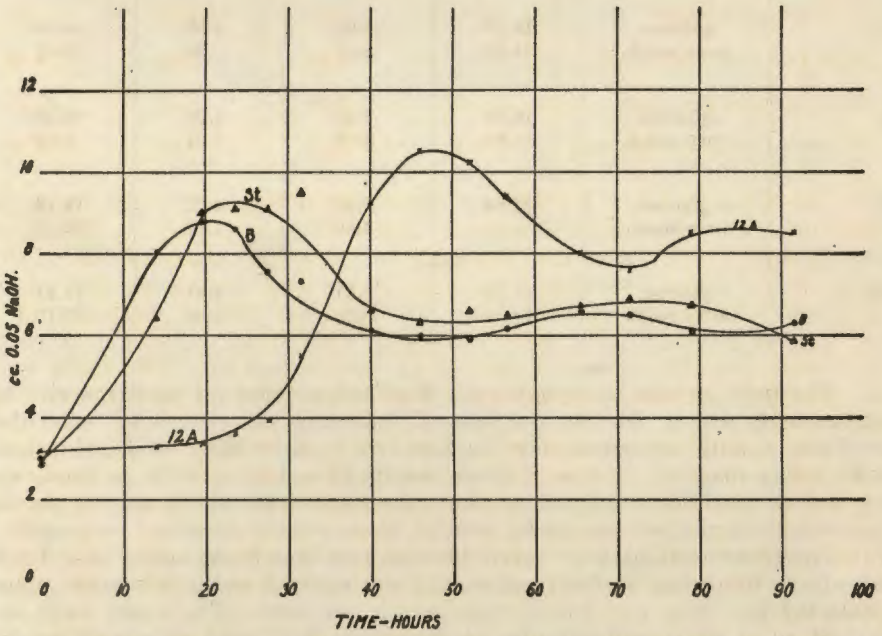


Fig. 1. Acid production by *Cl. acetobutylicum* from glucose in Robinson's medium.

It is difficult to advance any satisfactory explanation for the contradictory findings. Those who have worked with these organisms are familiar with the occasional wide variations which sometimes occur in two fermentations started with aliquots of the same original culture and carried out under conditions identical in all determinable respects. Thus, one flask may show the typical break in acidity while the second maintains an abnormally high value, or one fermentation may be vigorously under way in six to eight hours while the second shows a preliminary lag phase of 24 to 48 hours. With such results, under conditions which are identical in so far as determinable, it would however, be illogical to assume no cause

for these variations or to believe that conditions are identical. It would seem necessary to conclude that accompanying such results, there is some condition or set of conditions, differing in the two flasks, which eluding our methods of detection, still plays an important role in the behavior of the organism. Any attempted explanation of these factors or their biological significance would be, at this time, in the realm of speculation. We may suggest, however, that some of the divergent results reported in the literature may find explanation in some such set of undetermined factors which may have been maintained, under certain conditions, throughout the entire course of an investigation.

TABLE 3. *'Solvents' production by four strains of Cl. acetobutylicum from glucose in Robinson's medium*

| Strain | Medium | 'Solvents' by salting out | Acetone | Alcohols by oxidation | |
|---|---|---|---|---|---|
| | | | | EtOH | BuOH |
| B | glucose | 19.16 | 6.26 | 4.05 | 14.38 |
| | corn mash | 14.60 | 5.46 | 1.94 | 13.01 |
| I | glucose | 16.83 | 7.25 | 4.03 | 16.33 |
| | corn mash | 11.60 | 4.04 | 1.01 | 9.69 |
| K | glucose | 17.83 | 6.87 | 4.22 | 14.79 |
| | corn mash | —— | 4.80 | 1.17 | 12.30 |
| St. | glucose | 18.15 | 7.31 | 4.81 | 17.21 |
| | corn mash | 14.10 | 5.18 | 1.05 | 13.15 |

The next results to be reported were taken from an earlier series of experiments set up for the purpose of following the course of 'solvents' production and accompanying factors such as acidity and oxidation-reduction potential. A few of these results have been chosen to illustrate the points just discussed and to show the variations which may occur in fermentations carried out under conditions as nearly identical as possible.

The fermentations were carried out in two-liter flasks containing 1,800 ml. of the following medium: glucose, 3 per cent, di-basic potassium phosphate 0.1 per cent, and Bacto peptone 0.5 per cent. The flasks were arranged so as to permit samples to be withdrawn aseptically and to determine the oxidation-reduction potentials. The inoculum consisted of 50 ml. of an actively gassing 15 to 20 hour culture of strain 14c in the above medium. 'Solvents' were determined by the Babcock cream-bottle method. Corn and gluten extracts were added as shown in the tables.

The data from two of these experiments have been collected in tables 4 and 5. An examination of these data brings out several interesting facts. In each case, the fermentation which gave low solvent yields was accompanied by a relatively low final pH and an acidity curve failing to show the characteristic break, whereas the fermentations giving normal yields of 'solvents' had a final pH above 4 in each case and the accompanying acid curves show a pronounced break. The acidity curves accompanying

the poor 'solvents' producing fermentations will be seen to resemble those obtained by Weinstein and Rettger (1932).

TABLE 4. *Titrable acidity, Eh and pH of glucose fermentations by strain 14c, with gluten extract added. Acetone extract from 72 gm. of gluten added to each*

| | 1. | | | | | 2. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time hours | Eh | pH | T.A. | Acetone | Butyl alcohol | Eh | pH | T.A. | Acetone | Butyl alcohol |
| 2 | +.089 | 6.1 | 0.6 | | | —.091 | 6.4 | 0.3 | | |
| 2.5 | | | | | | | | | | |
| 4 | +.043 | | | | | | | | | |
| 5 | | | | | | —.114 | 6.4 | 0.3 | | |
| 6 | —.030 | 6.1 | 0.6 | | | | | | | |
| 7.5 | | | | | | —.114 | | | | |
| 8 | —.074 | | | | | | | | | |
| 9 | | | | | | —.239 | 6.3 | 0.3 | | |
| 10 | —.162 | 5.95 | 0.65 | | | | | | | |
| 12.5 | | | | | | —.288 | 5.2 | 0.5 | | |
| 13 | —.220 | 5.5 | 0.7 | | | | | | | |
| 16 | —.227 | 4.75 | 1.0 | 0.17 | 0 | —.278 | 4.4 | 1.0 | | |
| 19 | —.207 | 4.2 | 1.3 | | | | | | | |
| 23 | —.220 | 4.0 | 1.5 | 0.08 | 0 | —.257 | 4.1 | 1.5 | | |
| 27 | —.215 | | | | | | | | | |
| 30 | | | | | | —.239 | 4.3 | 1.3 | | |
| 32 | —.186 | 3.9 | 2.2 | 0.57 | 0 | | | | | |
| 36 | | | | | | —.244 | 4.5 | 0.9 | | |
| 41.5 | —.182 | 3.75 | 2.3 | | | —.248 | 4.5 | 0.9 | | |
| 48 | —.169 | 4.0 | 2.3 | 0.57 | 0 | —.248 | 4.4 | 0.9 | 7.40 | 11.42 |
| 54.5 | —.167 | 3.7 | 2.3 | 1.59 | 0 | —.242 | 4.5 | 0.9 | | |
| 63 | | | | | | —.086 | 4.5 | 1.0 | 7.56 | 13.94 |
| 67 | —.070 | 3.7 | 2.3 | 3.41 | 2.86 | | 4.4 | 1.0 | | |
| 76.5 | | | | | | —.086 | | | | |
| 97 | | | | | | —.047 | | | | |
| 130 | | | | | | —.041 | 4.1 | 1.0 | 8.32 | 20.42 |

It will be remembered that Wynne (1931) reported complete inhibition of the butyl-acetone fermentation resulted from high acidities represented by the pH range of 3.9 to 3.65. In the above data it will be observed that in both of the poor fermentations the pH had fallen below 3.9 before the end of 40 hours. Comparing these fermentations with the good 'solvents' producing fermentations it is seen that in the latter case the appearance of acetone and butyl alcohol is practically limited to the period between 40 hours and the end of the fermentation. This relation suggests the probability that a pH less than 3.9 has an inhibitory effect upon the enzymes involved in the reduction of the acids to alcohols.

A further examination of tables 3 and 4 shows a correlation between the oxidation-reduction potentials during the course of the fermentation and the 'solvents' production. In both cases where the production was good, the organisms were able to develop a negative Eh more rapidly and maintain it at a lower level for a longer period of time than fermentations responsible for the low 'solvents' production. As has been previously mentioned, there appears to be no satisfactory explanation for these variations in fermentations which should be identical.

Since the preliminary experiments indicated that normal 'solvents' production could take place from glucose in a semi-synthetic medium, it

TABLE 5. *Titrable acidity, Eh and pH of glucose fermentations by strain 14c, with corn extracts added. Butyl alcohol extract from 72 gm. of corn added to each*

| | 1. | | | | | 2. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time hours | Eh | pH | T.A. | Acetone | Butyl alcohol | Eh | pH | T.A. | Acetone | Butyl alcohol |
| 2 | +.243 | | 0.6 | | | | | | | |
| 2.5 | | | | | | —.244 | | | | |
| 4.43 | +.143 | | | | | | | | | |
| 5 | | | | | | —.300 | 5.4 | 0.4 | | |
| 6 | +.034 | 6.3 | 0.6 | | | | | | | |
| 7.5 | | | | | | —.245 | | | | |
| 8 | +.103 | | | | | | | | | |
| 9 | | | | | | —.222 | 4.5 | 1.0 | | |
| 10 | —.001 | 6.3 | 0.6 | | | | | | | |
| 12.5 | | | | | | —.211 | 4.5 | 1.3 | | |
| 13 | —.197 | 5.95 | 1.5 | | | | | | | |
| 16 | —.233 | 4.75 | 1.0 | | | —.215 | 4.1 | 1.4 | | |
| 19 | —.198 | 4.25 | 1.3 | | | | | | | |
| 23 | —.189 | 4.25 | 1.3 | 0 | 0 | —.198 | 4.2 | 1.6 | 0.34 | 0 |
| 27 | —.187 | | | | | | | | | |
| 30 | | | | | | —.177 | 4.3 | 1.4 | | |
| 32 | —.191 | 4.4 | 1.9 | 0.57 | 0 | | | | | |
| 36 | | | | | | —.185 | 4.3 | 1.3 | | |
| 41.5 | —.141 | 3.8 | 2.0 | 0.34 | 0 | —.196 | 4.4 | 1.1 | | |
| 48 | —.059 | 3.9 | 2.3 | | | —.192 | 4.4 | 1.0 | 4.70 | 6.05 |
| 54.5 | +.018 | 3.75 | 2.5 | 0.57 | 0 | —.167 | 4.4 | 1.0 | | |
| 63 | | | | | | —.162 | 4.4 | 1.0 | 7.73 | 13.77 |
| 67 | +.203 | 3.75 | 2.3 | 1.01 | 5.26 | | | | | |
| 76.5 | | | | | | —.043 | 4.5 | 1.0 | | |
| 97 | | | | | | +.113 | | | | |
| 130 | | | | | | +.148 | 4.5 | 1.0 | 9.91 | 18.76 |

appeared desirable to determine to what extent the other results reported by Weinstein and Rettger could be duplicated, or to determine if possible, the conditions leading to the production of normal quantities of acetone accompanied by little or no alcohol.

Weinstein and Rettger have taken exception to results reported by Peterson, Fred and Schmidt (1924), who obtained normal yields of 'solvents' from glucose, arabinose and xylose in a semi-synthetic medium. Weinstein and Rettger suggested that the normal fermentations met with there were possibly due to the introduction of sufficient zein with the corn mash inoculum. To test the correctness of this suggestion the following experiment was carried out. Strains B, I, K and St were transferred serially four times through tubes of Robinson's medium containing 3 per cent glucose. The fourth tube of each culture (in duplicate) was used as the inoculum for 400 ml. fermentations of glucose in Robinson's medium with corn mash controls. The results are shown in table 6.

It will be seen that the 'solvents' yields from glucose in Robinson's medium are comparable with those derived from corn mash and practically the same as those shown in table 3 where the media were inoculated with corn mash cultures. Since passage of the inoculum through four successive tubes of glucose-peptone medium eliminates all but traces of corn zein, it is evident that the yields cannot be explained by the introduction of such an alcohol soluble protein with the inoculum.

Weinstein and Rettger have reported further that the removal of the alcohol-soluble proteins from corn by extracting repeatedly with alcohol

and using the extracted corn as a substrate, resulted in normal yields of acetone while the alcohol yield dropped to from two to four per cent as compared with an average of about 13 per cent for the corn mash controls. We have repeated these experiments and failed to obtain the type of results reported by these investigators.

TABLE 6. *'Solvents' production from glucos in Robinson's medium following four successive transfers of the inoculum in glucose medium*

| Strain | Medium | Alcohol by salting out | Acetone | Alcohols by oxidation | |
|---|---|---|---|---|---|
| | | | | EtOH | BuOH |
| B | glucose | 17.34 | 6.07 | 3.07 | 16.34 |
| | corn mash | 12.50 | 6.00 | 0.84 | 13.66 |
| I | glucose | 16.17 | 6.51 | 4.57 | 15.27 |
| | corn mash | 15.64 | 6.82 | 1.53 | 14.67 |
| K | glucose | 19.82 | 6.95 | 3.56 | 17.47 |
| | corn mash | 14.10 | 6.38 | 1.83 | 13.65 |
| St. | glucose | 16.50 | 6.91 | 4.41 | 16.56 |
| | corn mash | 17.10 | 6.60 | 1.14 | 14.78 |

Yellow corn meal was extracted continuously for 24 hours with ethyl ether, followed by repeated extractions with 85 per cent ethyl alcohol by decantation. Nine extractions were made; each addition of alcohol was allowed to remain in contact with the corn meal for 12 hours before decantation. The last two extractions were colorless and the resulting corn meal was white. The extracted corn meal, after drying was made into five per cent mash and flasks of this medium along with normal corn mash controls were inoculated with 24 hour cultures of strains B, I, K, and St. After four days at 37°C. the fermentations were analyzed for 'solvents'. The results are shown in table 7.

TABLE 7. *'Solvents' production by Cl. acetobutylicum in alcohol-extracted corn*

| Strain | Medium | Alcohol by salting out | Acetone | Alcohols by oxidation | |
|---|---|---|---|---|---|
| | | | | EtOH | BuOH |
| B | extracted corn | 12.30 | 4.92 | 1.61 | 11.22 |
| | corn mash control | 14.25 | 6.18 | 1.14 | 15.93 |
| I | extracted corn | 11.67 | 5.14 | 1.39 | 12.66 |
| | corn mash control | 15.45 | 6.59 | 1.23 | 15.27 |
| K | extracted corn | 12.80 | 4.83 | 2.37 | 12.60 |
| | corn mash control | 11.35 | 5.42 | 1.60 | 13.49 |
| St. | extracted corn | 13.85 | 5.82 | 2.04 | 13.38 |
| | corn mash control | 13.35 | 6.69 | 0.96 | 15.26 |

The data in table 7 show some differences between the fermentation of the unextracted corn and that which was previously extracted with alcohol. Fermentation of the alcohol extracted corn gave a total 'solvents' yield somewhat lower than the corn mash control. The acetone and butyl alcohol yields are lower while the ethyl alcohol is slightly higher than with the unextracted corn. With the exception of these minor variations, however, the results are comparable and would be considered typical of *Cl. acetobutylicum* fermentation.

Since it appears that the formation of acetone and alcohols are to some extent, at least, independent processes, the possibility that the addition of toxic compounds to the fermentation might lead to a greater inhibition of one process than the other was considered. To test this suggestion, four one-liter flasks, each containing 600 ml. of Robinson's medium with 3 per cent glucose were inoculated with 20 ml. of a 24 hour corn mash culture of strain St. The acidity was followed in each flask, and immediately following the break in the acid curve (about 36 hours in all cases), flasks 2, 3 and 4 were treated respectively with one ml. of croten aldehyde, one ml. of chloroform and one ml. of toluene. Gassing and the head disappeared in less than two hours in the flasks receiving chloroform and toluene while the one receiving croten aldehyde showed vigorous gassing for about five hours following the addition. After four days incubation aliquots of the liquor were taken for the determination of 'solvents'. The results are shown in table 8.

TABLE 8. *'Solvents' production in glucose-peptone medium. Protoplasmic poisons were added 36 hours after start of fermentation*

| No. | Treatment | Acetone percentage | Alcohols by oxidation | |
|---|---|---|---|---|
| | | | EtOH percentage | BuOH percentage |
| 1 | Control | 6.84 | 2.18 | 19.82 |
| 2 | Croton aldehyde | 7.03* | 2.93 | 11.55 |
| 3 | Chloroform | 7.92* | 1.49 | 16.06 |
| 4 | Toluene | 5.92 | 1.63 | 13.06 |

* High acetone value due to chloroform and croton aldehyde.

The results show that, although the total 'solvents' yields are considerably lower in fermentations receiving additions of toxic substances, the acetone-alcohol ratio is not greatly changed.

In order to test the utilization of water insoluble proteins as a source of nitrogen, five per cent casein, and egg albumin and zein were added to flasks containing 400 ml. Robinson's inorganic medium with three per cent glucose. The flasks were inoculated with strain I and incubated for four days. Two glucose-peptone controls were run simultaneously. Although the controls gave normal yields of 'solvents' there was only a slight production in the flasks containing the water-insoluble proteins as nitrogen source, although each of the latter showed a transitory fermentation lasting about 24 hours.

During the autoclaving of the above protein media it was apparent that a certain amount of hydrolysis had taken place. This suggested the possibility that the transitory fermentations observed with these proteins might have arisen from the utilization of small amounts of hydrolytic products arising during autoclaving. To determine whether these organisms are able to utilize simple amino acids, hydrolysates of the above proteins were prepared by refluxing the various proteins for 36 hours with five times their weight of sulfuric acid (1 to 3). The resulting solution was

freed of sulfuric acid by adding an excess of calcium carbonate and filtering off the precipitated calcium sulfate. The filtrates were concentrated to 200 ml. and aliquots added to flasks containing Robinson's inorganic medium and three per cent glucose. Typical results of these fermentations are shown in table 9.

TABLE 9. *Solvents production from glucose with protein hydrolysates as the source of nitrogen*

| Strain | Nitrogen source | Acetone | Alcohols by oxidation | |
|---|---|---|---|---|
| | | | EtOH | BuOH |
| 1. | 5 percent hydrolized casein | 7.80 | 2.28 | 15.92 |
| St | 3 per cent hydrolized casein | 1.54 | 0.80 | 1.95 |
| St | 3 per cent hydrolized albumin | 7.34 | 1.03 | 15.27 |
| St | 2 per cent hydrolized albumin | 2.16 | 1.76 | 9.47 |
| St | 5 per cent hydrolized zein | 1.30 | 0.72 | 4.36 |
| St | 2 per cent hydrolized zein | 1.70 | 3.87 | 4.09 |
| St | Peptone control | 7.29 | 4.73 | 17.29 |

The results have not been consistent when amino acid mixtures were used as the only source of nitrogen. Further work, on the conditions accompanying the utilization of amino acids may offer an explanation, while the relatively high yields occasionally found are, at least, positive indication that such amino acid mixtures are capable of serving as nitrogen sources for the butyl organism.

## SUMMARY AND CONCLUSIONS

Fermentations have been carried out with seven strains of *Clostridium acetobutylicum* which have been systematically studied in this laboratory. Analyses of fermentations of glucose produced in the preliminary experiments have shown the production of normal yields of 'solvents' and that the organisms used were typical *Cl. acetobutylicum*. In Robinson's semi-synthetic medium with three per cent glucose all strains gave characteristic titrable acidity curves showing a pronounced drop following the peak. In the same medium, consistently good yields of 'solvents' have been obtained. All attempts to secure acetone-alcohol ratios such as those reported by Weinstein and Rettger (normal acetone yields with little or no alcohol) have failed.

In those occasionally occurring abnormal fermentations, in which low yields of 'solvents' are found, the fermentation is accompanied by factors which deviate from the normal, such as a failure of the acidity to break and a final pH lower than 3.9. Further, it appears that the normal fermentation is accompanied by a more reduced oxidation-reduction potential than the low 'solvents' producing fermentation.

Attempts to bring about the fermentation of glucose in Robinson's inorganic medium with such water insoluble proteins as egg albumin, casein

and zein serving as the only source of nitrogen were not successful. When hydrolysates of these proteins were used as the nitrogen source the results were inconsistent. In conclusion, it appears that some factor or group of factors, other than those commonly studied and controlled, plays an important part in determining the course of the butyl-acetone fermentation.

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# THE EFFECT OF DRY-CLEANING AND MECHANICAL CLEANING UPON FURS[1,2]

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This paper is a progress report of research which is being done by the Department of Textiles and Clothing on the effect of mechanical cleaning and dry-cleaning upon furs.

A limited amount of research has been done on the cleaning of furs and as far as it has been possible to determine, no work has been reported on the deterioration of the hair as the result of cleaning.

Goldman and Hubbard (2) of the United States Bureau of Standards experimented with the dry-cleaning of eight different furs; namely, beaver, fox, squirrel, Hudson seal, caracul, wolf, muskrat and raccoon. Two specimens of each of the furs were cleaned with naphtha and two with naphtha and paraffin. Each group was run for ten minutes, then extracted in a centrifuge and dried. After the cleaning, the appearance of the second group was good. The ether-soluble content of the cleaned furs and of the two untreated specimens was obtained. All of the "fat-free" specimens were next cleaned with a solution of paraffin and naphtha in one to forty proportion. The appearance of the furs was not injured. The specimens were again extracted with ether to determine the amount of fat which had been replaced by the paraffin. The results indicated that the loss in ether soluble content was small except in the case of raccoon.

Sowerwine (6) studied the effect of dry and mechanical cleaning upon ten different furs. Her results showed, in the case of dry-cleaning—using the formula worked out by Goldman and Hubbard, an increase in gloss due to the use of the paraffin and, an average decrease in the breaking strength and elasticity of the furs. There was practically no change in the mechanically cleaned furs.

According to Bachrach (1), who has examined thousands of specimens, the interior physical structure of hair and fabric undergoes few changes as the result of ordinary trade procedure. This structure is usually broken down by rough treatment.

Hausman (3) finds that external friction frequently causes alteration

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[1] A digest of five theses submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science, Iowa State College.

[2] The furs for testing were contributed by the Globe and Cownie Tanning Companies, Des Moines, Iowa, and Willard Fur Company, Marshalltown, Iowa. The mechanical cleaning was done by the Globe Tanning Company and the dry-cleaning by Wilson-Lindquist, Ames, Iowa.

[3] The authors wish to express their appreciation for helpful criticism of the manuscript to the following professors of Iowa State College: Dr. Rachel Edgar, Department of Textile Chemistry, Dr. E. R. Becker, Dr. G. O. Hendrickson and Dr. J. E. Guthrie, Department of Zoology, and Dr. Louise Peet, Department of Household Equipment; and to Dr. William Kunerth, Department of Physics, for instruction in the use of the reflectometer.

in the form of scales. Usually the scales at the base of the hair are of greater longitudinal and transverse diameter than those at the tip of the shaft. The changes in scales are usually found in protective hairs rather than in the under hairs of the pelt.

Hausman's (5) experiments show that "the hair shaft consists of four structural units: (1) the medulla or pith, built up of many shrunken and varioiusly disposed cells or chambers representing dried and cornified epithelial structures connected by a branching filamentous network, which sometimes completely fills the medullary column, but which is interrupted in many cases; (2) the cortex or shell of the hair shaft surrounding the medulla composed of elongated fusiform cells or hair spindles, coalesced together into a horny almost homogeneous, hyaline mass and forming in many cases, where the medulla is reduced, a large proportion of the hair shaft; (3) the pigment granules to which the color of the hair is primarily due (though in some hairs the pigment is diffuse and not granular in form) scattered about within or between the hair spindles and in some hairs arranged in definite patterns; and (4) the cuticle, or outermost integument of the hair shaft lying upon the cortex, and composed of imbricated, thin, hyaline colorless scales of varying forms and dimensions. It is the forms, relationships and measurements of these four elements together with the measurements of the diameter of the hair shaft itself in micra which constitute the series of determinative criteria for each species of hair."

Hausman's (4) microscopic study of the hair shows that it is strengthened by the cortex and that the greater the cortex in proportion to the medulla the greater is the resistance of the hair to wear. The medulla is an element of weakness in the composition of the hair shaft, being composed of empty or practically empty cells, frequently with disconnected strands of elastic substance running between them. Therefore, if the medulla is large in proportion to the cortex, the hair is nearly a hollow cylinder and is easily broken. As soon as the scales of a hair are worn off the cortex is soon injured and the hair shaft deteriorates rapidly. With hairs having large medullas, wear begins much earlier than it does in hairs having small or no medullary columns.

Hausman discovered by testing fur with an attritometer that unclipped furs are superior to clipped furs. Hair which has not been clipped does not wear until after the hair breaks or portions of the protecting cuticle are injured by wear.

There is some variation in the cortex of the hair of different species of fur-bearing mammals which makes it less resistant than the hair of other mammals. The quality of the keratin of the cortex cells and their fusion probably accounts for this variation.

## EXPERIMENTAL

Ten furs were selected for experimental purposes; namely, Hudson Seal, Northern Seal, opossum, skunk, muskrat, beaver, squirrel, rabbit, fox and raccoon. Salt alum tannage was used for the furs and the specimens tested were taken from the backs of the pelts and matched in quality as closely as possible.

For the sake of accuracy the controls in each of the four sets were averaged for each test. The following tests were run on the furs in allotment I during the year 1930-1931.

The ether-soluble content of each of the ten different furs in the con-

trol and cleaned groups was determined by ether extraction, and the average deviation from the control was calculated. The furs were heated to constant weight at 105°C., extracted with anhydrous ether in a Soxhlet extractor for eighteen hours, air-dried, and heated to constant weight again. The weighings were made by the method of tares.

Shrinkage was determined by blocking off a one-inch square and measuring it before and after cleaning.

The thickness of the skin was determined by means of a micrometer. Ten determinations from each of the ten kinds of fur were made from both the control and cleaned furs, and the average deviation from the control was calculated.

The diameter of the hair was determined on both control and cleaned furs by means of a micrometer. For each kind of fur, ten determinations were made and the averages calculated.

Change in the color of the dyed furs, caused by cleaning, was determined by the use of a reflectometer. In each case four determinations were made and the averages calculated.

Change in gloss, caused by the use of paraffin in dry-cleaning, and Polar Bear Meal in mechanical cleaning, was determined by means of a glarimeter. Three readings were taken with the flow of the hair going upward and three with the flow going downward. Averages were determined and the deviation from the control calculated.

The number of hairs to the square inch was determined by pulling out hairs and counting. Three determinations with averages were made of hair from each kind of fur.

Length of hair was determined by measuring separately with a linear steel scale both the guard hairs and the under hairs. Ten determinations of each were made and averages calculated.

Breaking strength and elasticity of the furs were determined by the strip method with the Scott Universal Tester (8). The specimens were taken from different portions of the pelt. The tests were run at a temperature of 71°F. and 80 per cent humidity. Five determinations were made of each of the ten kinds of fur in both the control and the cleaned groups, and the average deviation from the control was calculated.

Wear, caused by abrasion, was determined on both control and cleaned furs with the Wyzenbeck Precision Wear Tester. The specimens were weighed air dry and given 1,500 double rubs, then weighed again immediately after testing. Three determinations of each of the different furs were made and the average loss in weight and the deviation from control calculated.

Dry-cleaning was done by the formula given in Technical Bulletin No. 35, National Association of Dyers and Clearners (7).

The specimens of fur were placed in the washer in a solution of Stoddard Dry-Cleaning Solvent, and run for ten minutes. As soon as the dirt was loosened, the fur was placed in a centrifuge and whirled for approximately one minute, long enough to extract all naphtha. The strips were then placed in a solution of paraffin in Stoddard Dry-Cleaning Solvent and allowed to soak for one minute, being gently manipulated to assure even penetration. A cotton cloth was placed in the paraffin solution to absorb any water which it might contain. The strips were again centrifuged for one minute. When removed, they were shaken well and hung in a ventilated dry-room at a temperature of 100°F.

Mechanical cleaning was done by putting the furs into a large drum,

called a cleaning drum, along with fur coats. Approximately five gallons of "Polar Bear Meal," a corn product, was placed in the drum with them. The furs were tossed in the cleaning drum for one and one-half hours, next placed in an open drum, and tossed for one and one-half hours in order to shake the meal from the fur. After cleaning, the furs were glazed by brushing the fur against the flow of the hair with a stiff brush which had been dipped into water and then shaken. The fur was next combed with a wire brush and hung on a rack, at room temperature. After the fur was dry, it was laid upon a padded table and beaten with bamboo sticks. This process removed any remaining cleaning meal and also fluffed up the fur, giving it a light airy appearance.

Microscopic examination of scales and medulla were made of both control and cleaned furs. Slides were made of scales and of both crosswise and lengthwise sections of guard hairs and under hairs.

In making slides of these sections the hairs were boiled for one-half hour in a fast red dye. Those to be used for cross sections were tied in bundles and cleared with xylol. To remove the water, they were run through chloroform and then through ether. The bundles of hair were then embedded in celloidin until ready to cut. They were next put into a mixture of 95 per cent ethyl alcohol and glycerin, in equal proportions until slightly softened.

These blocks were then cut in the microtome into sections measuring from fifteen to twenty microns. The cut sections, except the cross sections of the under hair, were put into 95 per cent alcohol and acetone to dissolve the celloidin. Carbol-xylol and lastly pure xylol was used to clear the sections before mounting them in balsam on the glass slides.

In order to see the scales more clearly, glycerin mounts were made of the hairs. These mounts were made by putting the hair into a solution of pure glycerin and absolute alcohol and allowing the mixture to evaporate to the consistency of pure glycerin over a waterbath. The slides were then made by putting a hair, together with some of the jelly, on a glass slide, and covering all with a cover glass which was cemented on with a cover-glass cement. Photomicrographs were made from the slides by means of a Leitz photomicrographic camera.

In the fall of 1931 the furs were taken from cold storage. One control, one mechanically cleaned and one dry-cleaned set were put aside for testing. The remaining furs with the exception of the controls were again cleaned, after which, one set of each of the mechanically cleaned and dry-cleaned furs was also put aside for testing, and the furs not tested were again placed in cold storage.

The tests which were used in 1930-1931 were repeated on the second allotment of furs in 1931-32 and on the third allotment in 1932-33. Each year deviations were determined from previous results.

## DISCUSSION OF DATA

In all cases the mechanically cleaned furs lost more than the dry-cleaned furs by ether extraction. The first dry-cleaning took the greater proportion of the fat from the specimens. This fact would indicate that the original ether-soluble content of the furs was not replaced after cleaning. In the case of every fur, the loss of fat from dry-cleaning was greater than from mechanical cleaning.

Raccoon showed a greater deviation in loss of fat than any of the

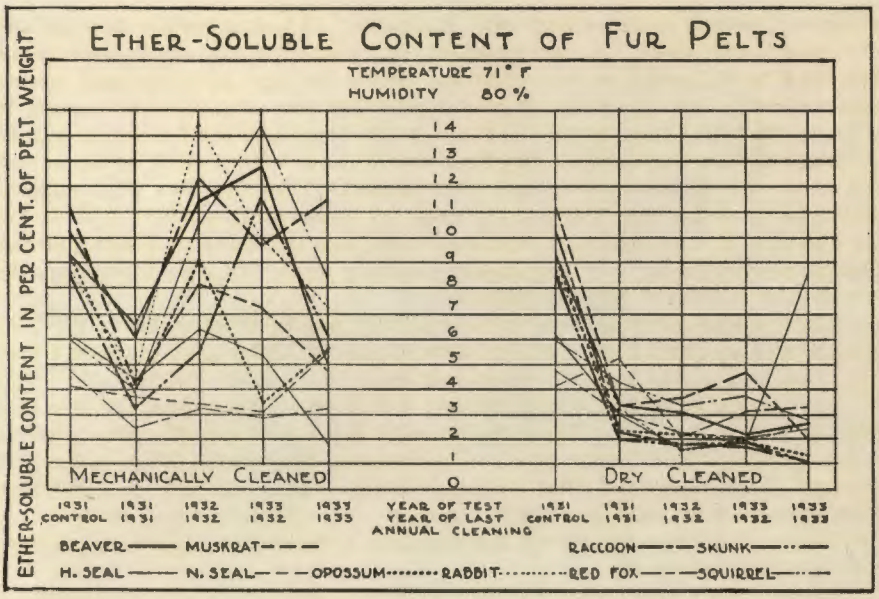


Fig. 1. Ether-soluble content of pelts.

furs. It also lost less weight than any of the other dry-cleaned specimens. Goldman and Hubbard (2) obtained similar results for raccoon.

In most cases the mechanically cleaned furs maintained a greater average breaking strength, but the difference was not pronounced except in the case of raccoon. Raccoon had the greatest average breaking strength of all the furs and Hudson seal had the least breaking strength. The average results in breaking strength were in favor of mechanical cleaning.

In general, the average elongation of fur was greater in the mechanically cleaned specimens. Raccoon showed greater variation in elongation in both processes than the other furs. With the exception of beaver, both mechanically cleaned and dry-cleaned furs showed a definite increase in elongation after the first cleaning, which dropped to nearly normal after storage and later cleanings.

Both mechanically cleaned and dry-cleaned furs showed a definite loss in thickness after the first cleaning, but they increased in thickness with later cleanings. Raccoon was the only fur to lose in thickness from both types of cleaning. The average thickness of all the furs was slightly greater for the mechanically cleaned furs than for the dry-cleaned furs.

The average loss in weight from abrasion was greater, in most cases, for the dry-cleaned furs than for the machanically cleaned furs. In all groups, the least average loss in weight was found in beaver, the greatest average loss in weight was found in opossum. In general, the soft-haired furs lost more in weight than the coarser furs which had a greater number of guard hairs.

The average loss in weight from abrasion after three cleanings was greater in the dry-cleaned furs than in the mechanically cleaned furs.

The average gloss of the dry-cleaned furs was higher than that of the mechanically cleaned furs.

Length of hair did not seem to be materially affected by cleaning.

TABLE 1. *Average percentage of color reflection of dyed furs with deviation from control*

| Kind of fur | Control 1931 | Tested in 1931 Cleaned in 1931 | | | Tested in 1932 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cleaned in 1931 | | | | Cleaned in 1932 | | | |
| | | | Deviation from control | | | Deviation from control | | | Deviation from control | | Deviation from 1931 | |
| | Reflection factor | Reflection factor | Difference | Pctg. | Reflection factor | Difference | Pctg. | Reflection factor | Difference | Pctg. | Difference | Pctg. |
| **Mechanically cleaned** | | | | | | | | | | | | |
| Hudson Seal | 0.01913 | 0.0170060 | —0.002124 | —11.10 | 0.01534 | —0.00379 | —19.8 | 0.017803 | —0.00133 | —6.9 | +0.000797 | +4.6 |
| Northern Seal | 0.01983 | 0.0181426 | —0.001687 | —8.51 | 0.02373 | —0.00390 | +19.7 | 0.020310 | +0.00048 | +2.4 | +0.002168 | +11.9 |
| **Dry cleaned** | | | | | | | | | | | | |
| Hudson Seal | 0.01913 | 0.024109 | +0.004979 | +26.03 | 0.02866 | +0.00953 | +49.8 | 0.03463 | +0.03463 | +81.02 | +0.01053 | +43.85 |
| Northern Seal | 0.01983 | 0.024838 | +0.005009 | +25.26 | 0.02653 | +0.00670 | +33.7 | 0.03613 | —0.01630 | +82.19 | +0.01130 | +45.13 |

TABLE 1. (Continued)

| Kind of fur | Tested in 1933 Cleaned in 1933 | | | | | Average readings of cleaned furs | Average of deviations |
|---|---|---|---|---|---|---|---|
| | Reflection factor | Deviation from control | | Deviation from 1932 | | | |
| | | Difference | Pctg. | Difference | Pctg. | Difference | Pctg. |
| **Mechanically cleaned** | | | | | | | |
| Hudson Seal | 0.003487 | —0.015643 | —81.77 | —0.014316 | —80.41 | 0.013409 | —29.89 |
| Northern Seal | 0.008845 | —0.010985 | —55.40 | —0.011465 | —56.45 | 0.017757 | —10.45 |
| **Dry cleaned** | | | | | | | |
| Hudson Seal | 0.015366 | —0.003764 | —19.68 | —0.01927 | —55.64 | 0.102765 | +34.29 |
| Northern Seal | 0.016977 | —0.002853 | —14.39 | —0.01916 | —53.03 | 0.026119 | +31.69 |

TABLE 2. *Deviation of average gloss of furs from year to year*

| Kind of fur | Control | Cleaned and tested in 1931 | Cleaned in 1931 and 1932 Tested in 1932 | | Cleaned in 1931, 1932 and 1933 Tested in 1933 | | | Average gloss of cleaned furs | Average deviation from control |
|---|---|---|---|---|---|---|---|---|---|
| | | Deviation from control | Deviation from control | Difference in deviation | Deviation from control | Difference in deviations | Difference in deviations | | |
| | Degrees | Percentages | Percentage | Percentage | Percentage | Percentage | Percentage | Degrees | Percentage |
| **Mechanically cleaned** | | | | | | | | | |
| Beaver | 12.07 | +24.9 | +24.4 | —0.5 | +38.2 | +13.3 | +13.8 | 15.59 | +29.2 |
| Red fox | 19.28 | —43.3 | +31.7 | +75.0 | —45.3 | —2.0 | —77.0 | 15.62 | —18.9 |
| Muskrat | 18.23 | —2.1 | +47.8 | +49.9 | +43.7 | +45.8 | —4.1 | 23.66 | +29.8 |
| Opossum | 21.04 | +6.7 | +4.1 | —2.6 | —35.4 | —42.1 | —39.5 | 19.32 | —8.2 |
| Rabbit | 25.45 | —0.7 | +5.2 | +5.9 | —27.8 | —27.1 | —33.0 | 22.60 | —11.2 |
| Raccoon | 19.58 | +17.4 | +24.1 | +6.7 | —33.6 | —51.0 | +57.7 | 24.48 | +2.6 |
| Hudson Seal | | | | | | | | | |
| Northern Seal | | | | | | | | | |
| Skunk | 33.32 | +6.9 | —16.9 | —23.8 | —29.2 | —36.1 | —12.3 | 28.98 | —13.1 |
| Squirrel | 33.36 | —29.9 | —12.8 | +17.1 | —13.4 | +16.5 | —0.6 | 27.12 | —18.7 |
| **Dry cleaned** | | | | | | | | | |
| Beaver | 12.07 | —6.4 | +163.9 | +170.3 | +51.4 | +57.8 | —112.5 | 20.47 | +69.6 |
| Red fox | 19.28 | —35.1 | +11.1 | +46.2 | +42.4 | +77.5 | +31.3 | 20.46 | +6.1 |
| Muskrat | 18.23 | +4.7 | +18.3 | +13.6 | +37.7 | +33.0 | +19.4 | 21.91 | +20.2 |
| Opossum | 21.04 | +99.6 | +32.3 | —67.3 | +29.1 | —70.5 | —3.2 | 32.33 | +53.6 |
| Rabbit | 25.45 | —2.4 | +0.3 | +2.7 | +37.1 | +39.5 | +36.8 | 28.42 | +11.6 |
| Raccoon | 19.58 | +25.2 | +2.7 | —22.5 | +55.3 | +30.1 | +52.6 | 25.01 | +27.7 |
| Northern Seal | | | | | | | | | |
| Hudson Seal | | | | | | | | | |
| Skunk | 33.32 | —12.4 | —30.7 | —18.3 | —27.5 | —15.1 | +3.2 | 25.47 | —23.5 |
| Squirrel | 33.36 | —19.3 | —15.8 | +3.5 | —14.4 | +4.9 | +1.4 | 27.85 | —16.5 |

Note: Gloss on Hudson Seal and Northern Seal could not be determined because it was impossible to get satisfactory reflection on the black furs.

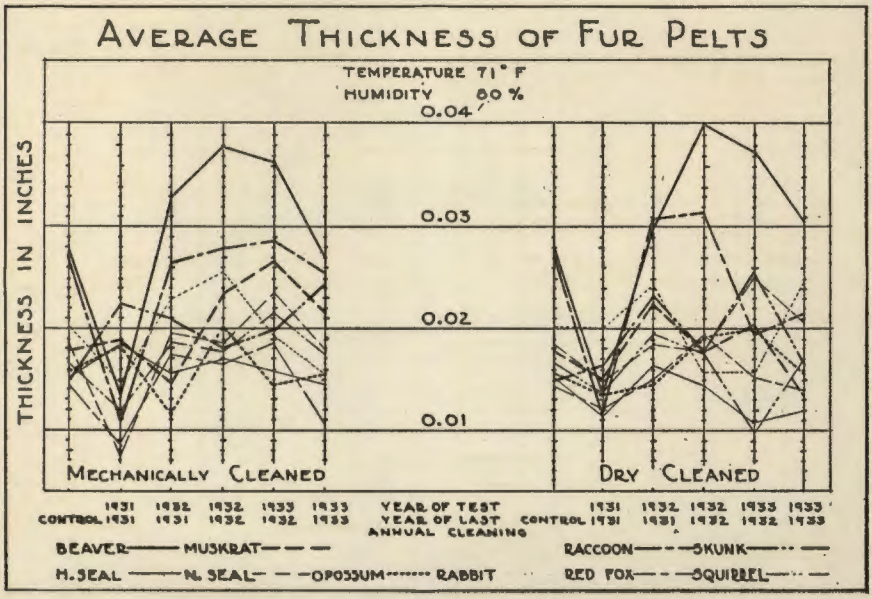


Fig. 2. Average thickness of fur pelts.

TABLE 3. *The number of hairs per square inch of pelt*

| Kind of fur | Guard hair | Under hair | |
|---|---|---|---|
| | Average | Average | Total |
| Beaver | 432 | 117,180 | 117,612 |
| Red fox | 1,589 | 39,525 | 41,114 |
| Muskrat | 3,605 | 102,218 | 105,823 |
| Opossum | 1,402 | 13,736 | 15,139 |
| Rabbit | 2,538 | 40,837 | 43,375 |
| Raccoon | 985 | 22,645 | 23,630 |
| Hudson Seal | 1,434 | 49,829 | 51,263 |
| Northern Seal | 645 | 34,933 | 35,578 |
| Skunk | 1,493 | 13,952 | 15,445 |
| Squirrel | 2,576 | 33,130 | 35,706 |

Note: The hair count was taken of both muskrat and Hudson Seal (dyed muskrat), also, of rabbit and Northern Seal (dyed rabbit).

The average diameter of hair tended to be slightly greater in dry-cleaned furs than in the mechanically cleaned furs.

In the mechanically cleaned furs, the average percentage deviation from control indicated a loss in diameter for all under hair except skunk, but the guard hair showed little variation from control.

The average reflection factor of dyed furs was greater in the dry-cleaned than in the mechanically cleaned furs.

The dry-cleaned furs appeared to have lost in pigment after cleaning; the greater proportion of the loss in pigment was evident after the first cleaning.

Six out of nine tests run on the furs were in favor of mechanical cleaning—ether-soluble content, breaking strength, elongation, thickness of pelt, abrasion, and length of hair. The increase in gloss, diameter and

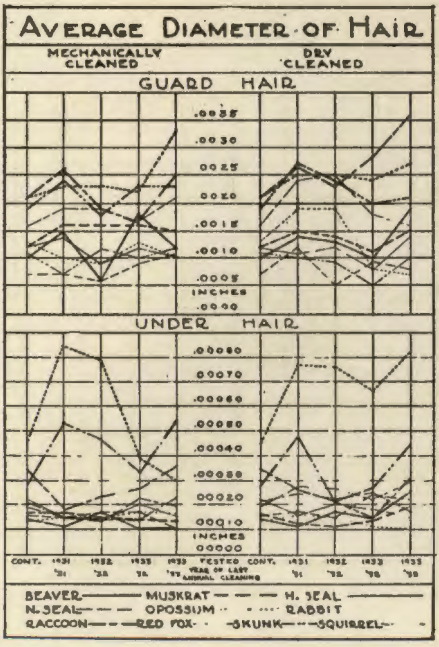


Fig. 3. Average diameter of hair.

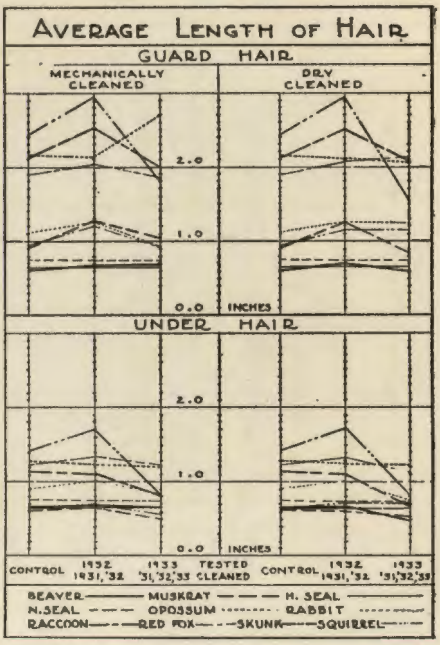


Fig. 4. Average length of hair.

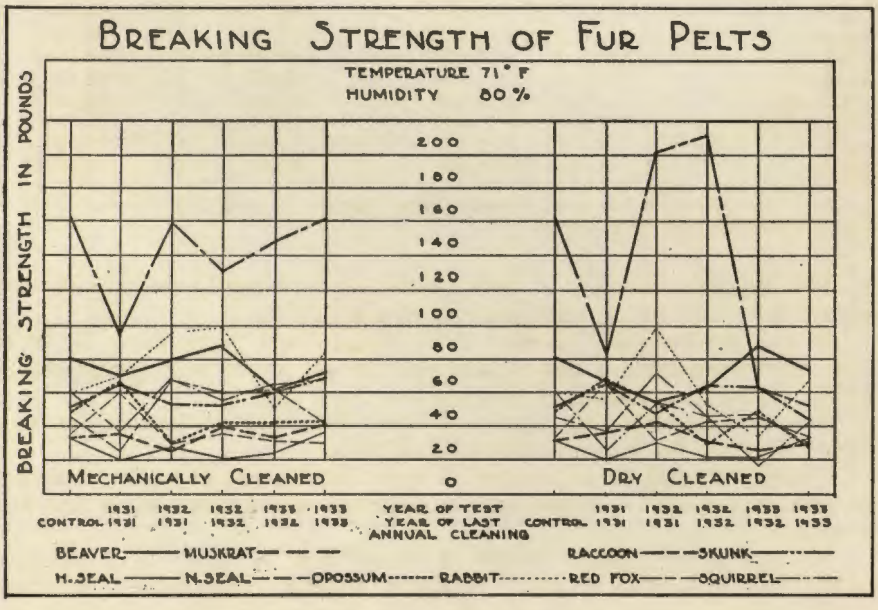


Fig. 5. Breaking strength of fur pelts.

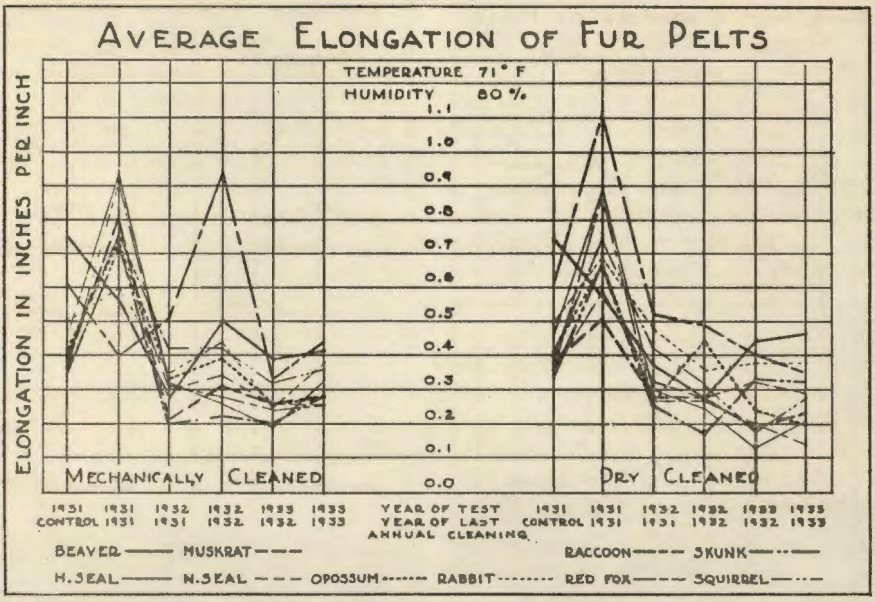


Fig. 6. Average elongation of fur pelts.

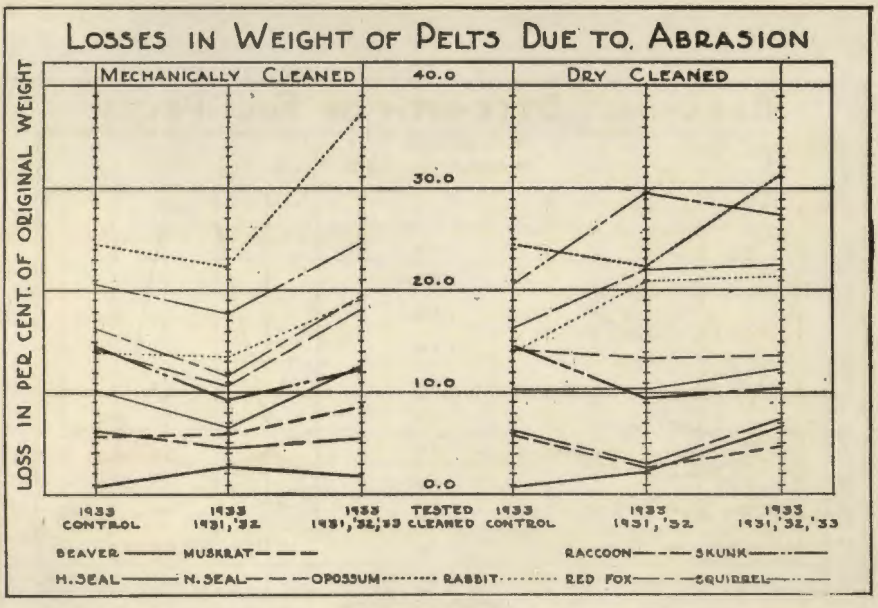


Fig. 7. Loss in weight of fur pelts due to abrasion.

reflection factor averages following dry-cleaning was without doubt caused by the paraffin oil rinse.

Examination of the photomicrographs of the medullas, cross-sections, and scales of hair when compared with the photomicrographs made in previous years show the effect of cleaning upon the hair structure of the furs.

Plates I-IV show photomicrographs of the hair structure of the furs.

1. The scales of dry-cleaned furs were less pronounced than those of the controls and mechanically cleaned furs. Also, the scales of the under hair were larger than those of the guard hair of the same fur.

2. The hair having the largest diameter had the smallest scales and the hair having the smallest diameter had the largest scales.

3. The less durable furs had large medullas and sharp scales. Beaver had the smallest medulla and rabbit the largest medulla, in proportion to the cortex of any of the furs studied.

4. A definite pattern was found in the medulla of each kind of fur.

5. The photomicrographs of clipped furs which were dyed showed that the dye penetrates the medulla as well as the cortex.

Hausman's experiments on fur confirm conclusions 2, 3, and 4 in relation to the hair structure of the furs.

## SUMMARY OF TESTS MADE

The furs, Hudson Seal, Northern Seal, opossum, skunk, muskrat, beaver, squirrel, rabbit, fox and raccoon, were tested for ether-soluble content, breaking strength and elasticity, shrinkage, color reflection, diameter of hair, gloss, number of hairs to the square inch, length of hair, loss in weight from abrasion; and a microscopic study was made of the hair of the control and cleaned furs.

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## EXPLANATION OF PLATES

Note: Photographs of longitudinal and cross section of medulla of the cleaned furs, also of the scales of the mechanically cleaned furs are not given since the structure of the hair showed no appreciable change.

### PLATE I

Fig. 1. Beaver.

Fig. 2. Red fox.

1A. Longitudinal section of medulla of guard hair of control. Magnification 500X.

2A. Cross section of medulla of guard hair of control. Magnification 250X.

1B. Longitudinal section of medulla of under hair of control. Magnification 500 X.

2B. Cross section of medulla of under hair of control. Magnification 250X.

1C. Scales of guard hair of control.

2C. Scales of guard hair after dry-cleaning.

1D. Scales of under hair of control.

2D. Scales of under hair after dry-cleaning.

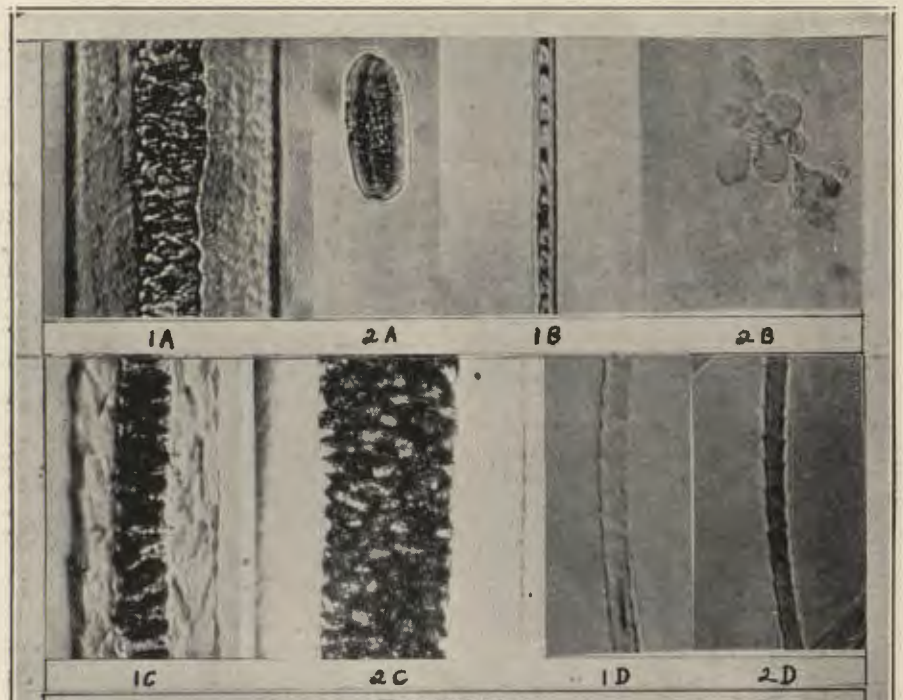


FIGURE 1

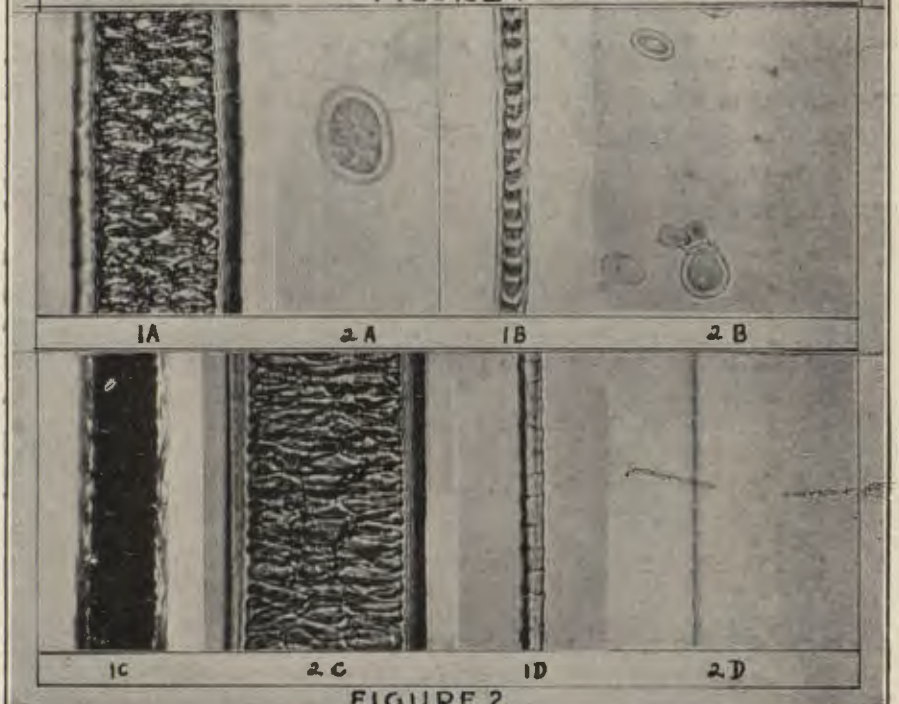


FIGURE 2

## PLATE II

Fig. 1. Muskrat.

Fig. 2. Opossum.

1A. Longitudinal section of medulla of guard hair of control. Magnification 500X.

2A. Cross section of medulla of guard hair of control. Magnification 250X.

1B. Longitudinal section of medulla of under hair of control. Magnification 500X.

2B. Cross section of medulla of underhair of control. Magnification 250X.

1C. Scales of guard hair of control.

2C. Scales of guard hair after dry-cleaning.

1D. Scales of under hair of control.

2D. Scales of under hair after dry-cleaning.

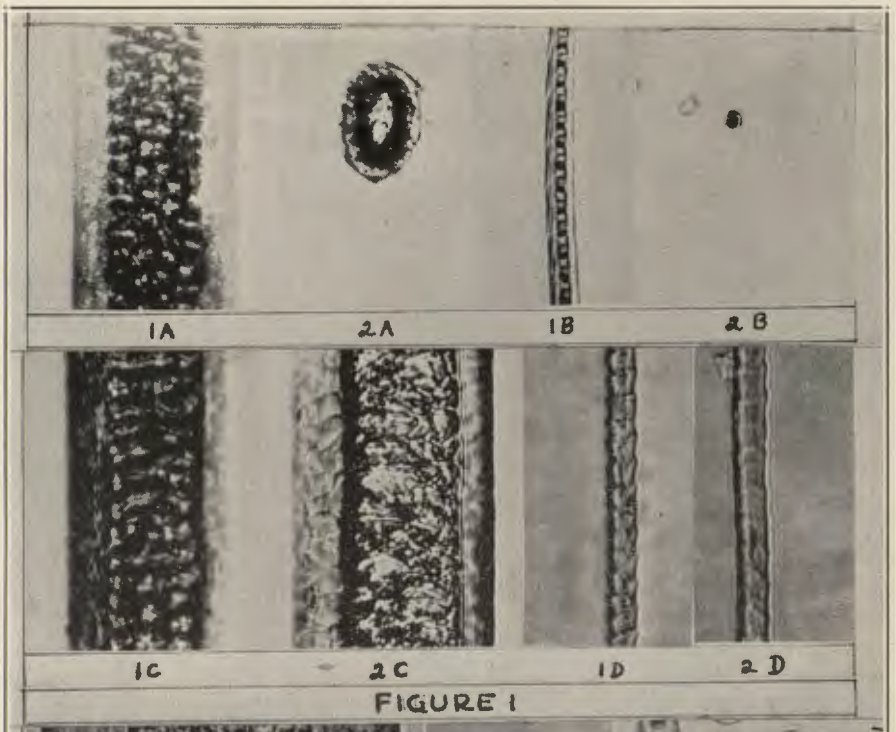


FIGURE 1

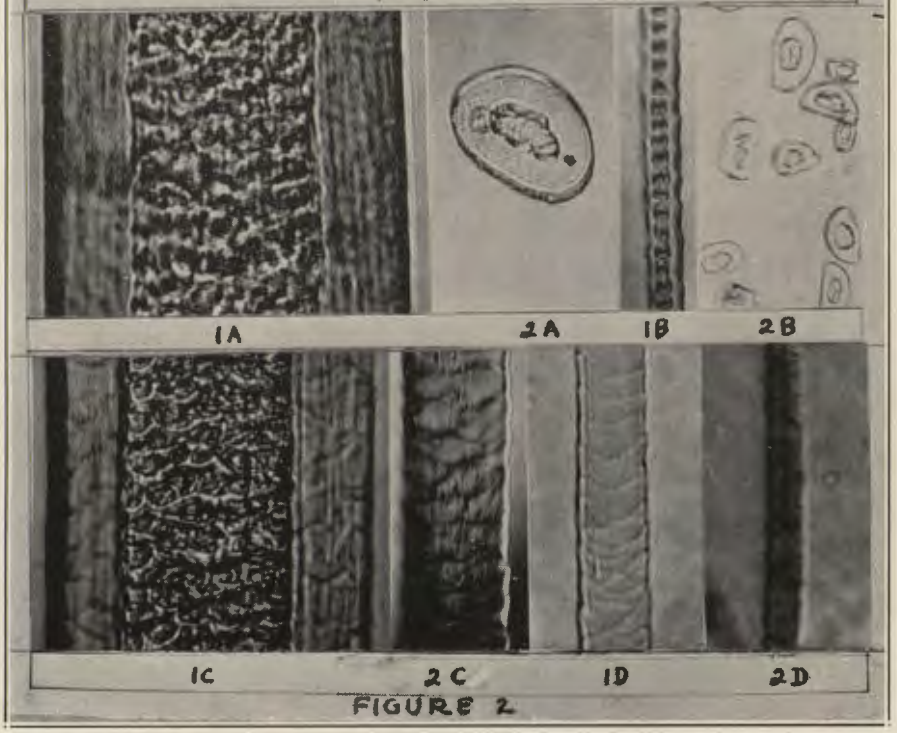


FIGURE 2

## PLATE III

Fig. 1. Rabbit.

Fig. 2. Raccoon.

1A. Longitudinal section of medulla of guard hair of control. Magnification 500X.

2A. Cross section of medulla of guard hair of control. Magnification 250X.

1B. Longitudinal section of medulla of under hair of control. Magnification 500X.

2B. Cross section of medulla of under hair of control. Magnification 250X.

1C. Scales of guard hair of control.

2C. Scales of guard hair after dry-cleaning.

1D. Scales of under hair of control.

2D. Scales of under hair after dry-cleaning.

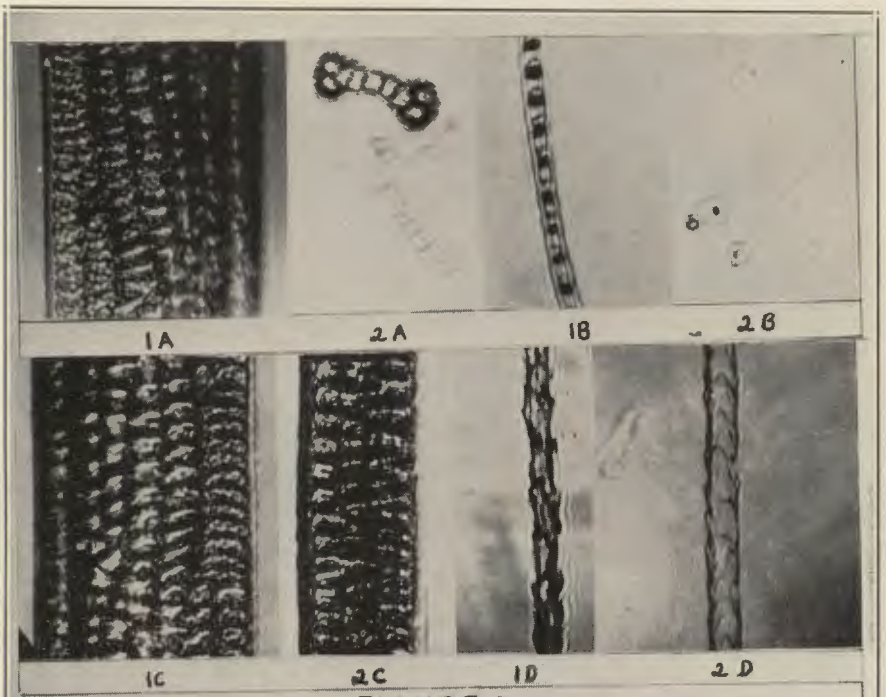


FIGURE 1

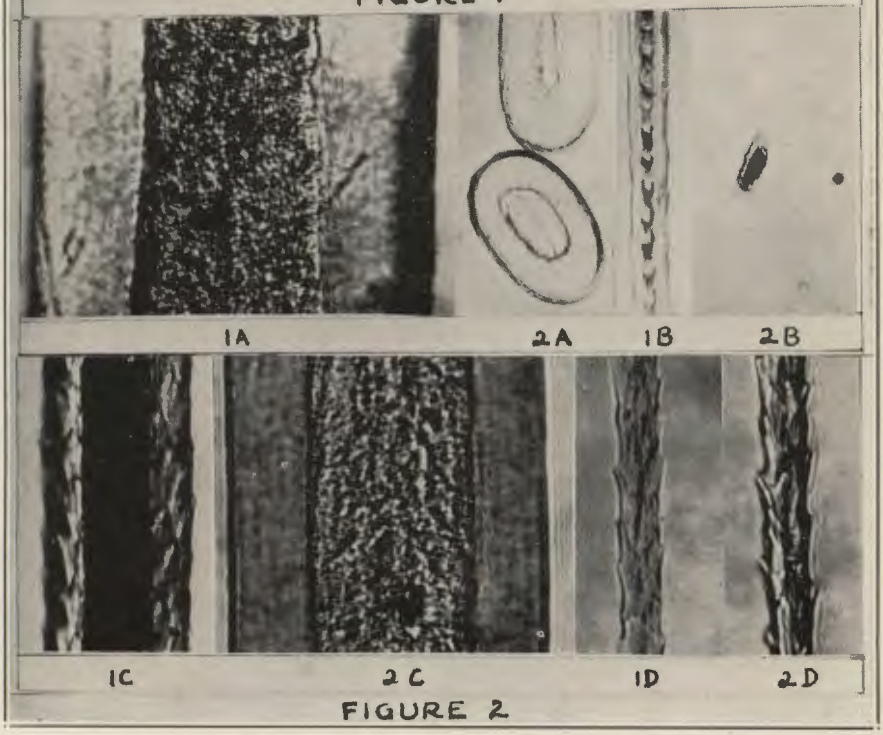


FIGURE 2

## PLATE IV

Fig. 1. Hudson Seal.

Fig. 2. Northern Seal.

1A. Longitudinal section of medulla of guard hair of control. Magnification 500X.

2A. Cross section of medulla of guard hair of control. Magnification 250X.

1B. Longitudinal section of medulla of under hair of control. Magnification 500X.

2B. Cross section of medulla of under hair of control. Magnification 250X.

1C. Scales of guard hair of control.

2C. Scales of guard hair after dry-cleaning.

2D. Scales of under hair after dry-cleaning.

2D. Scales of under hair after dry-cleaning.

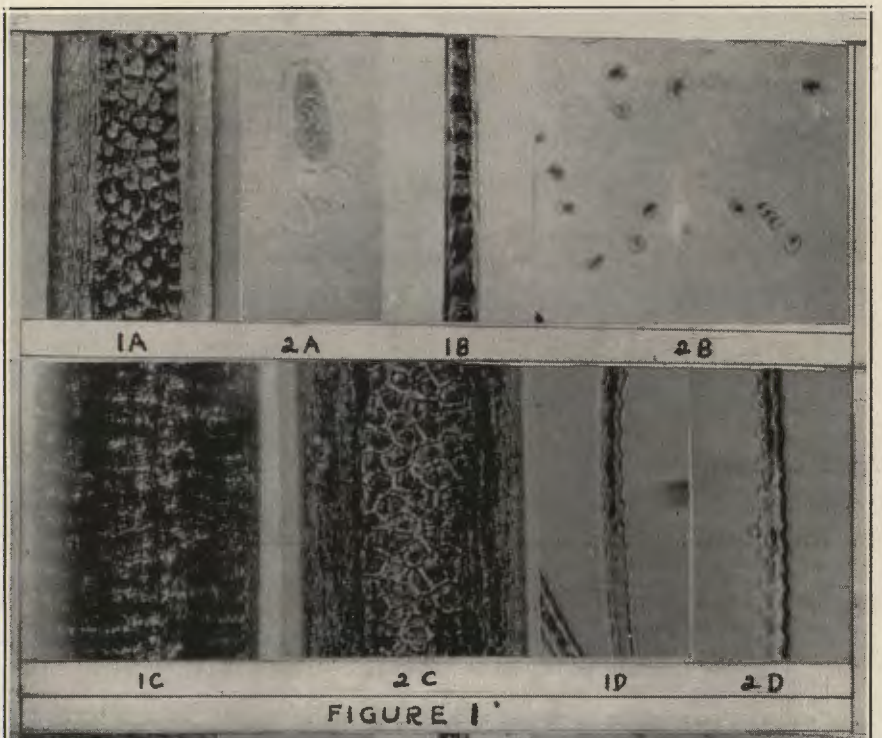


FIGURE 1

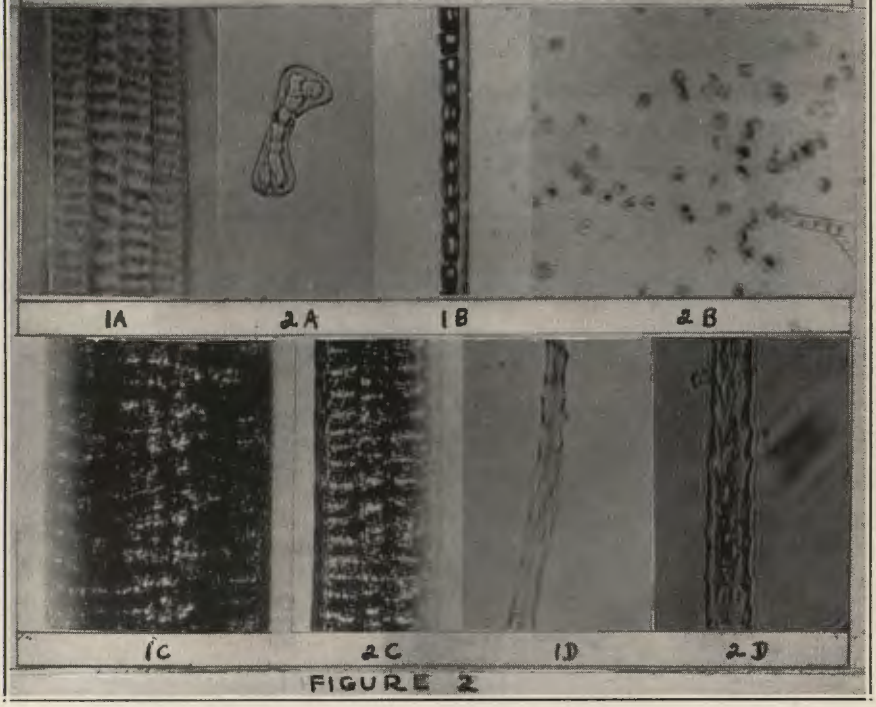


FIGURE 2

## PLATE V

Fig. 1. Skunk.

Fig. 2. Squirrel.

1A. Longitudinal section of medulla of guard hair of control. Magnification 500X.

2A. Cross section of medulla of guard hair of control. Magnification 250X.

1B. Longitudinal section of medulla of under hair of control. Magnification 500X.

2B. Cross section of medulla of under hair of control. Magnification 250X.

1C. Scales of guard hair of control.

2C. Scales of guard hair after dry-cleaning.

1D. Scales of under hair of control.

2D. Scales of under hair after dry-cleaning.

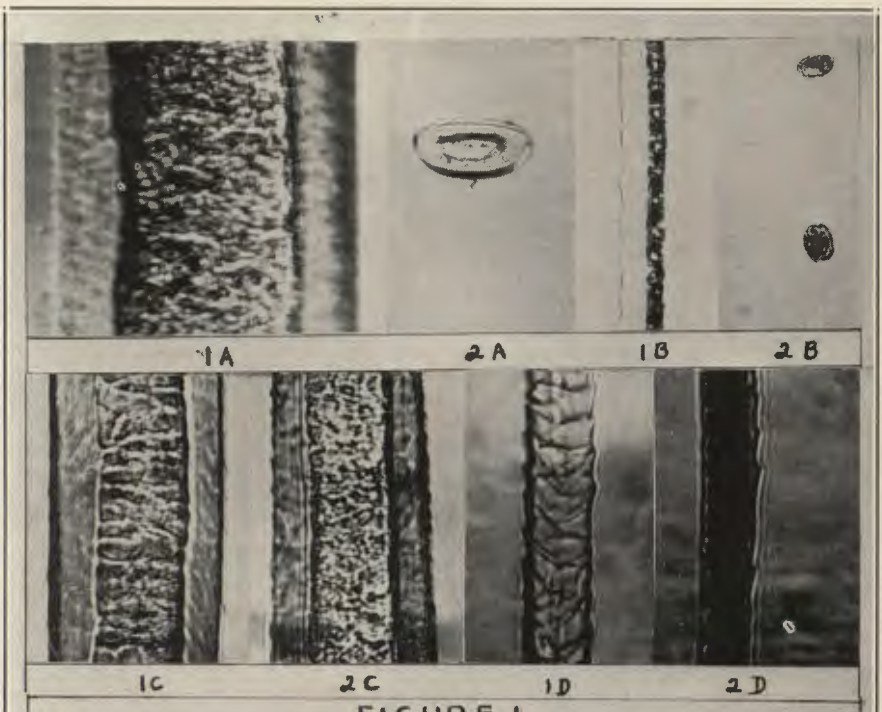


FIGURE 1

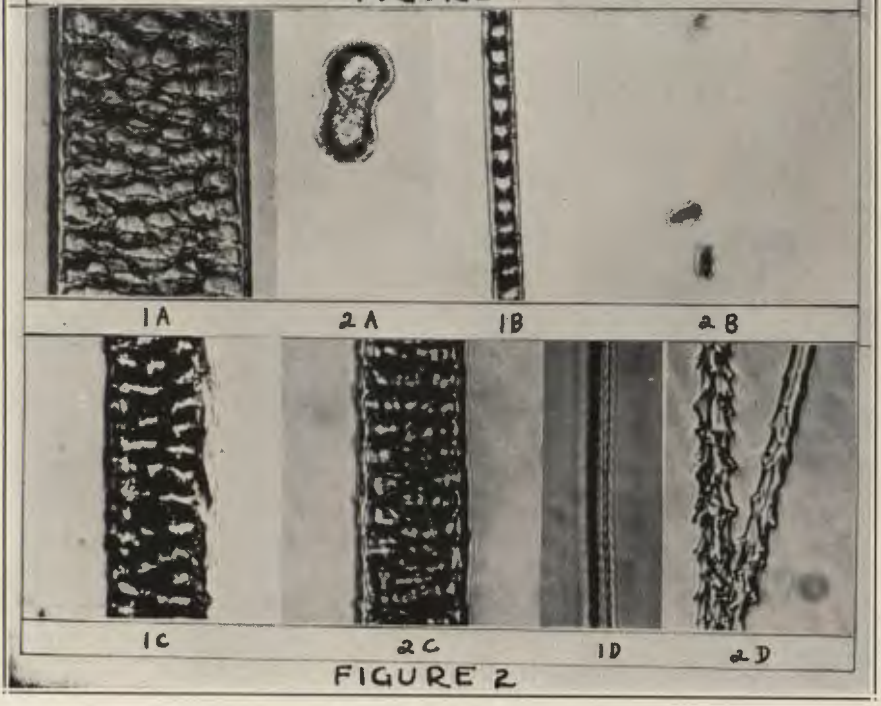


FIGURE 2

# THE PLASMODESMS IN THE LEAVES AND STEMS OF SOME ANGIOSPERMS

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The investigations herein reported were undertaken to ascertain the prevalence and nature of the plasmodesms[1] in the leaves and stems of a number of our common plants, and with the further object of securing some information relative to the functioning of the plasmodesms in the inter-cellular conduction of stimuli, plant foods, viruses, and various substances, even including disease-producing organisms or agents.

In the Cell Theory, as announced by Schleiden and Schwann (1838), multicellular organisms are regarded as aggregates of morphologically separate, independent cells. This conception, with some modifications, still maintains a prominent place in biology, notwithstanding its criticism by Sachs, De Bary, Strasburger, and other able proponents of the Organismal Theory. In contrast to the Cell Theory, the Organismal Theory, as stated by Sachs (Vorlesungen uber Pflanzen physiologie, p. 102), holds that the multicellular organism, however highly organized, is fundamentally a protoplasmic body consisting of a connected whole which is externally clothed by a cell membrane and internally traversed by innumerable transverse and longitudinal walls.

One of the strongest supports of the conception that the protoplasm in multicellular organisms is a connected whole is the presence of connective-like structures between adjacent protoplasts, a feature that has been noted by a large number of investigators and in many species of plants. That these connective-like structures are protoplasmic, and that they are generally continuous, thus establishing a continuity between adjacent protoplasts, have not, however, been fully substantiated and by some authors have been considerably discredited. In as much as their universal presence between the cells of the various living tissues has not been thoroughly established and their continuity is questioned, the plasmodesms can not be evaluated as to their place in the protoplasmic system of multicellular organisms.

Two general types of these apparent connections have been recognized; the relatively large ones, such as those in the sieve tubes and laticiferous vessels of seed plants, and in some species of the red algae; and the fine strands, called Plasmodesmen by Strasburger, which usually require special methods for their detection, a feature that may be responsible for a lack of appreciation of their importance. While the larger connections are regarded as quite limited in occurrence, the plasmodesms have been observed in nearly all living tissues throughout the large groups of plants. Owing

[1] The use of *plasmodesms* instead of *plasmodesmen* is according to Sharp's "Introduction to Cytology." 3rd ed. 1934. McGraw-Hill, New York.

to their wide occurrence the plasmodesms are of greater significance than the large connections in relation to the protoplasmic continuity of multicellular organisms. The establishment of their universality in living plant tissues and the fact that they are protoplasmic continuities functioning as passageways between adjacent protoplasts, as Sachs, Kienitz-Gerloff (8,9), Gardiner (2,3), Strasburger (12), and some other prominent authorities have maintained, not only would facilitate the explanation of the spread of solutes, viruses, stimuli, and probably disease-producing organisms through tissues, but would account also for the cooperative activities of tissues, such as occur in many of the ordinary physiological processes of plants and in the formation of graft unions and in all other phenomena where the plant functions more or less as a unit.

From the date of Tangl's (13) report in 1879 (claimed to be the first account of plasmodesms) till 1902, plasmodesms attracted the attention of many investigators. Their work disclosed the presence of plasmodesms in the algae, fungi, liverworts, mosses, ferns, Gymnosperms, and Angiosperms.

## METHODS AND MATERIALS

The plants included in this investigation were mainly common economic plants, many of which are subject to virus disturbances. Monocotyledons and dicotyledons–both woody and herbaceous types–were represented. The particular plants included were the Irish potato, tomato, pepper, egg plant, nasturtium, watermelon, cucumber, field pumpkin, hubbard squash, onion, regal lily, corn, one species of aster, several varieties of apples, shagbark hickory, and white oak.

Owing to the disclosure that the method of demonstrating the presence of plasmodesms in the leaves and stems did not work so satisfactorily when applied to the tissues of roots, the investigations here reported have been confined to the stems and leaves.

All the preparations were made from fresh material. For the study of plasmodesms in epidermal tissues, mounts of portions stripped from the leaves and stems sufficed. All sections were made free-hand with the aid of a binocular. Satisfactory sections were limited to one cell in thickness.

The satisfactory demonstration of plasmodesms entails (a) the killing of the protoplasts to prevent excessive shrinking during ensuing treatments, (b) the hydration of the cell walls in order to increase their transparency and separate the protoplasts that the plasmodesms may be more visible, and (c) the staining of the protoplasts and plasmodesms without staining the cell walls. In regard to the killing solutions, hydrating reagents, and stains, many different successful methods have been employed of which Tunmann (14) gives a good summary. The reagents which have been used most commonly are: iodine for a killing reagent, as it also assists in the staining of the plasmodesms; chlor-zinc-iodide or sulfuric acid for hydrating the walls; and pyoktanin, methyl violet, gentian violet, haematoxylin or safranin as a staining reagent.

A comparison of the results obtained by the various methods given in Tunmann's Pflanzenmikrochemie, lead to the selection of a modification of Myer's method in which iodine was used for killing and mordanting the tissues, sulfuric acid for hydrating the walls, and pyoktanin for staining the plasmodesms and protoplasts. The sections were placed in a water

solution of iodine two or three minutes, then in 25, 50, and 72 per cent sulfuric acid, remaining only a few minutes in each. Since the rate of action of the acid depends much upon the character of the tissues, a number of trials often were required to determine the proper concentration of acid to use and the time it should be allowed to act. When the hydration was considered sufficient, as indicated by the dark blue to black color of the preparations, the acid was removed by filter paper and pyoktanin then applied and allowed to act five to 10 minutes, after which the preparations were thoroughly rinsed in water and in acetic or weak sulfuric acid when the excess stain was difficult to remove. The chief object of the destaining was to secure a transparency of cell walls. The preparations were finally mounted in 50 per cent glycerine. The pyoktanin used was a freshly made saturated water solution.

While the swelling of the walls accompanying their hydration contributed much to the visibility of the plasmodesms, it resulted in a stretching of the plasmodesms which probably in some instances altered their diameter considerably; and when it was carried far the plasmodesms were broken by the tension as may be seen in a number of the illustrations. No effort was made to ascertain quantitatively how much the plasmodesms were altered by the swelling of the walls. However, in those preparations where different amounts of swelling were obtained, no marked difference in size of plasmodesms traversing walls differently swollen was noted.

## PLASMODESMS IN LEAVES

In the leaves the plasmodesms were most easily observed in the epidermal tissue, owing in part to the relative ease with which preparations of suitable thickness could be obtained, and in part to the size of the plasmodesms, some of which were 60 or more microns in diameter in the swollen condition of the tissues of the preparations. The plasmodesms in the mesophyl of leaves were delicate, and for their detection thin transverse sections of fresh material were required. The demonstration of their presence, especially between the palisade cells and between the epidermal and mesophyl cells, was rather difficult and consequently was confined to a few of the species included. Where preparations failed to show the presence of plasmodesms between some of the cells in the mesophyl, the unfavorable orientation and differences in staining reactions of the plasmodesms between different cells were perhaps responsible.

Between the elongated rectangular epidermal cells, such as are characteristic of grass leaves and are common in the region of the veins of most leaves, two types of plasmodesms were noted. Those traversing the end walls were numerous, fine, and in their size, regular arrangement, and in the barrel-shape figure they formed resembled the fibers in mitosis (fig. 8), whereas those traversing the side-walls commonly occurred singly or in small groups, varied in diameter from a few to 60 or more microns and were irregularly distributed (figs. 2, 3, 4, 8). In the leaves of some plants, as those of corn (fig. 4), the lateral connections were relatively numerous, whereas in the leaves of some other plants, as illustrated by the onion (fig. 2), they were comparatively sparse. Similar variations were found in *Viscum album* by Kuhla (11) who reported a variation ranging from 26 to 46 in the number of lateral plasmodesms between the epidermal cells of the leaf.

The plasmodesms between the irregularly shaped epidermal cells, although varying considerably in size and arrangement, were of one type (figs. 1, 5, 6) and resembled the lateral connections between the rectangular epidermal cells. In a surface view they appeared relatively numerous and pretty well distributed through all the transverse walls, except those bordering the guard cells, where, according to the casual observations, they were more delicate, fewer in number and larger, or lacking, all these situations sometimes occurring in the same preparation. A more careful study, however, would likely have disclosed- that the plasmodesms are constantly present between the guard cells and adjacent epidermal cells though quite variable in type, at least for different species of plants. Such a disclosure would be in accord with the observations of both Kohl (10) and Kuhla (11) who reported the presence of plasmodesms between the guard cells and adjacent epidermal cells as a constant feature in *Viscum album.*

In general the plasmodesms in the epidermal cells of the leaves and in all the tissues having thin cellulose walls in both leaves and stems were observed to traverse the walls as undivided strands throughout their length. They were the solitary type according to Kuhla's (11) classification, which designates those undivided in their course as solitary plasmodesms, whereas those divided into fine strands in a part of their course are designated as the aggregate type.

It was relatively easy to demonstrate in thin longitudinal sections of veins a system of plasmodesms establishing connections between the epidermal cells and the tissues of the veins and between the different tissues of the veins. In the leaves of the potato, onion, and aster, where special effort was made to demonstrate their presence in the mesophyl, plasmodesms were found connecting the epidermal cells with the palisade and spongy parenchyma. Those plasmodesms establishing connections between the palisade cells and epidermal cells were delicate and sparse while those between the palisade cells in the preparations observed were delicate but relatively numerous. These observations are in part out of accord with Kuhla's (11) report that in the leaves of *Visrum album* the plasmodesms between the epidermal and palisade cells were notably large. Those observed between the spongy cells were quite variable in size, some being delicate and others relatively large (fig. 7).

## THE PLASMODESMS IN STEMS

### IN THE PRIMARY MERISTEM

Since the primary tissues of stems, such as those constituting the first formed epidermis, cortex, phloem, cambium, xylem, and rays, are immediate products of the primary meristems, the presence of plasmodesms in the primary meristems was of special interest. In the stem tips of both the herbaceous and woody stems, the presence of plasmodesms was easily demonstrated (figs. 9, 10, 11), thus disclosing that the stem tissues were formed from cells between which there was already a well-established system of plasmodesms. This raises the question as to the history of the plasmodesms in connection with the processes of differentiation that transform the cells from the meristem into the various types of tissues. The plasmodesms of the tip meristems in all the stems observed were comparatively uniform in the various aspects. Between some cells they were fine and numerous, re-

sembling the system of fibers in mitosis while those radiating from the same cells in other directions were often few but comparatively large (figs. 10 and 11). In the tip meristems observed in the herbaceous stems most, if not all, of the plasmodesms were of the solitary type; whereas in the tip meristems of woody stems it was noted, especially in the basal region of the meristems, that some of the large plasmodesms were divided into fine strands near midway of their course. This feature was found to be most pronounced in meristems in winter dormancy (fig. 11).

### IN THE TISSUES OF THE OLDER REGIONS OF STEMS

Between the epidermal and sub-epidermal cells of stems the presence of plasmodesms was readily demonstrated and between the cells of the cortex the plasmodesms were especially pronounced (figs. 13-18). Thus the epidermal cells were found to communicate laterally with each other and internally with the cortex. The cells of the cortex were connected in all directions by plasmodesms. In preparations, such as those made from lengthwise radial sections, which are specially favorable for showing the plasmodesms through the tangential and transverse walls, it was possible to trace an uninterrupted series of plasmodesms from epidermis through the cortex and even into the xylem when sections of suitable extent and thickness could be made. Where bark was present they were traceable only from the cork cambium. The observations as to their presence in cork tissue were rather casual; but, if present, they were either less conspicuous than those of the cells beneath or required different methods to make them visible.

In both radial and tangential lengthwise sections, in which views the cells of stems, except those of the rays, are generally rectangular and usually much elongated in the direction of the axis of the stem, two rather distinct types of plasmodesms were observed (fig. 14). Those traversing the end or transverse walls were numerous, fine, and regularly arranged. They resembled the fibers in mitosis. Those transversing the lateral walls were irregularly distributed, quite variable in size, and much less numerous per unit area of wall surface than those traversing the end walls. They were either single or in small groups. In size they ranged from a few to 65 microns in diameter. In stems, especially in herbaceous ones, the plasmodesms were similar as to types and general features to those previously described between the rectangular cells in surface views of the epidermal tissues of leaves and stems. In woody stems where the walls were thickened the lateral plasmodesms were commonly of the aggregate type, being divided into delicate strands, variable in number, in the region of the middle lamella (fig. 14). Also when the walls in herbaceous stems were considerably thickened, as, for example, in the flowering stalk of the onion, the lateral plasmodesms were similarly divided into fine strands through the region of the middle lamella. Apparently this feature of the plasmodesms is associated with wall thickening and does not depend upon the species or the type of plant.

In the rays, especially in woody stems, the plasmodesms were exceptionally pronounced (fig. 19). They were present not only between adjacent ray cells but between ray cells and adjacent parenchyma cells of the xylem and phloem running lengthwise of the stem (fig. 20). It was

thus possible in stems to trace a system of plasmodesms which had its origin in the primary meristem and had so developed during subsequent growth as apparently to maintain connections between all the living cells. The origin of the plasmodesms in the meristems and their establishment of a complete system of connections between the living cells have been previously described in a number of Angiosperms by Kienitz-Gerloff (8, 9), Kuhla (11), Strasburger (12), Gardiner (2, 3), and others and in *Pinus sylvestris* and allied species by Gardiner (3) and Hill (4). This, of course, does not establish the fact that such a system of connections consists of protoplasmic continuities at all points in its course, thus maintaining as a whole the protoplasm of the entire stem, as has been the belief of a number of authorities. There still remains the task of establishing the protoplasmic nature and continuity of the plasmodesms.

In those herbaceous stems where the plasmodesms were single throughout their course, the evidence was much in favor of the theory of protoplasmic continuity, for the plasma membranes and contents of the plasmodesms appeared as continuations of the protoplasts. There were no indications of interruptions between the protoplasts they connected (fig. 3). In cases where the plasmodesms are divided into fine threads during a part of their course, like those characteristic of thick walls, there was no evidence of a discontinuity between the plasmodesms and the protoplasts (fig. 12). The question of continuity here pertains to the fine strands whose continuity was difficult to trace. Mention should be made here of recent articles by Jungers (6, 7) who is inclined to regard plasmodesms as structures of the cell wall and therefore not protoplasmic. His conclusions were based on studies of plasmodesms in endosperms, sieve calluses, and in some of the red algae. It seems that if his idea is correct there should be recognizable breaks or lines of juncture between the plasmodesms and the protoplasts, and such was not the case in the material observed in this investigation. In the plasmodesms that are divided into delicate strands in the region of the middle lamella, as those characteristic of thick walls, the junctures in the fine strands may easily escape detection. However, in the onion, apple, and spruce, where effort was made to decide this question, the evidence favored the view that the continuity was maintained throughout the entire length of the plasmodesms.

A number of functions, such as the conduction of stimuli, enzymes, and various types of substances, have been ascribed to the plasmodesms. Judged upon their prevalence, apparent size and continuity, the simple plasmodesms, which were found to be characteristic of the herbaceous plants and of tissues with thin walls in general are capable of affording passage ways not only for stimuli and solutions, but for bodies many times the size of most disease-producing agents or organisms. In case of the aggregate type of plasmodesms the fine strands were commonly visible under the ordinary high-power combinations of the microscope. Their size would therefore permit the passage of small micro-organisms, and in the conduction of solutions their number may compensate for their lack in size.

The fact of the apparent ever-presence of the plasmodesms between the cells in the primary meristems and throughout the subsequently formed tissues of the stems and leaves, suggests that they have a place of importance in the protoplasmic organization of the plant. Strasburger (12) noted that plasmodesms were formed between stock and scion of grafts in the

apple, spruce, and fir and considered that they have an important bearing in the establishment of good unions. Buder (1) and Hume (5) have contributed support to Strasburger's contention, and the prevalence, structural features, and history of the plasmodesms disclosed in this article strongly favor it.

## SUMMARY

The investigation pertains to the prevalence and nature of the plasmodesms in the leaves and stems of the Irish potato, tomato, pepper, egg plant, nasturtium, watermelon, cucumber, field pumpkin, hubbard squash, onion, regal lily, corn, one species of aster, several varieties of apples, shagbark hickory, and white oak.

A satisfactory procedure for making the plasmodesms visible in leaves and stems consisted of killing the material in iodine, hydrating the walls in sulfuric acid, and staining the protoplasts and plasmodesms with pyoktanin.

In all the plants included in the observations, plasmodesms were present in all the living tissues examined and apparently between all the living cells of the leaves and stems.

The plasmodesms were of two general types; those traversing the walls as single strands, and those divided into finer strands in a part of their course. The type consisting of a single strand was characteristic of leaves, herbaceous stems, and in general of tissues with thin walls, whereas the second type of plasmodesms was characteristic of tissues with thick walls and hence of woody stems. Between the elongated rectangular cells of herbaceous tissues there were usually two kinds of the simple or solitary type of plasmodesms. Those traversing the end walls were numerous, fine, evenly spaced, uniform in size, and formed barrel-shaped figures resembling those of the spindle fibers in mitosis, whereas those traversing the side walls were irregularly distributed, varied in size from a few to 65 or more microns in diameter and were commonly single but often in small groups.

In the simple type of plasmodesms the evidence of continuity was quite convincing, as no discontinuity in plasma membrane or in protoplasmic contents was detectable anywhere in the course of the plasmodesms. In the other type of plasmodesms, where there was a division into fine strands that traverse the closing membrane of the pit, it was difficult to decide whether or not the fine strands were continuous. In the onion, spruce, and apple, however, by special effort it was possible to so trace the fine strands so as to convince one of their continuity.

The plasmodesms were found present in all the primary meristems of stems. Thus all the tissues arising therefrom, as those of stems, leaves, and flowers, are products of cells primarily characterized by the presence of plasmodesms. It was possible to trace a continuous series of plasmodesms which had its origin in the primary meristems and embraced all subsequent tissues arising therefrom. In herbaceous plants where it was evident that the plasmodesms maintain a continuity of protoplasm, their prevalence supports the Organismal Theory. Owing to their prevalence and size throughout their entire course in leaves and stems of herbaceous plants and in the thin-wall tissues in all plants, it is quite believable that plasmodesms function in the conduction of viruses, organisms, plant foods, and

various other types of substances. In tissues with thick walls, such as are characteristic of woody plants, the adaptability of the plasmodesms to conduct is less apparent because so many of them are divided into fine strands whose continuity is not obvious, and whose capacity to conduct is apparently small. Nevertheless, if continuous, the fine strands are large enough to permit small disease-producing organisms or agents to pass, and their number may compensate for their lack in size in their conduction of solutions.

## ACKNOWLEDGMENT

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## PLATE I

### EXPLANATION OF FIGURES

The plasmodesms in all the following figures are the dark strands between the dark bodies which are the protoplasts. The stem sections are all lengthwise radial.

Fig. 1. Surface view of some epidermal cells of an Irish potato leaf, showing the plasmodesms; x 450.

Fig. 2. Surface view of some rectangular leaf cells of an onion leaf showing variation in size and the sparseness of the plasmodesms; x 450.

Fig. 3. Surface view of some rectangular leaf cells of an aster between which the plasmodesms are rather numerous, and vary in size from a few to as many as 40 microns in diameter; x 450.

Fig. 4. Surface view of the epidermis of a corn leaf showing the plasmodesms rather numerous and quite variable in size; x 450.

Fig. 5. Surface view of some cells of a pepper leaf showing the plasmodesms; x 450.

Fig. 6. Surface view of a number of cells of a pepper leaf giving a more comprehensive view of the number and distribution of the plasmodesms; x 200.

Fig. 7. Some spongy cells of a pepper leaf showing at the point of the arrow the delicate connections common between the spongy cells of leaves; x 800.

Fig. 8. A rectangular epidermal cell from a leaf of the Irish potato showing the large lateral connections and at the ends of the arrows the fine plasmodesms that resemble the fibers in mitosis; x 800.

Fig. 9. A general view of the plasmodesms in the meristem of an apple twig; x 200.

Fig. 10. Enlarged view of some cells from the meristematic tip of an apple twig showing prevalence and variability of the plasmodesms; x 450.

Fig. 11. Some cells from the base of a meristematic tip of an apple twig showing the plasmodesms a little better differentiated into large scattered lateral ones and the fine, numerous end ones; x 450.

Fig. 12. An enlarged single plasmodesm showing its continuity with the protoplasts at each end; x 800.

Fig. 13. Section through the cortex of an apple twig showing the plasmodesms; x 320.

Fig. 14. A section through the cortex of an apple twig where the cells were elongated rectangles, showing the large lateral connections, many of which are divided into fine strands through the region of the middle lamella and the fine plasmodesms through end walls; x 600.

Fig. 15. Plasmodesms in the cortex of the stem of an Irish potato; x 450.

Fig. 16. Plasmodesms in tho cortex of a Norway spruce; x 200.

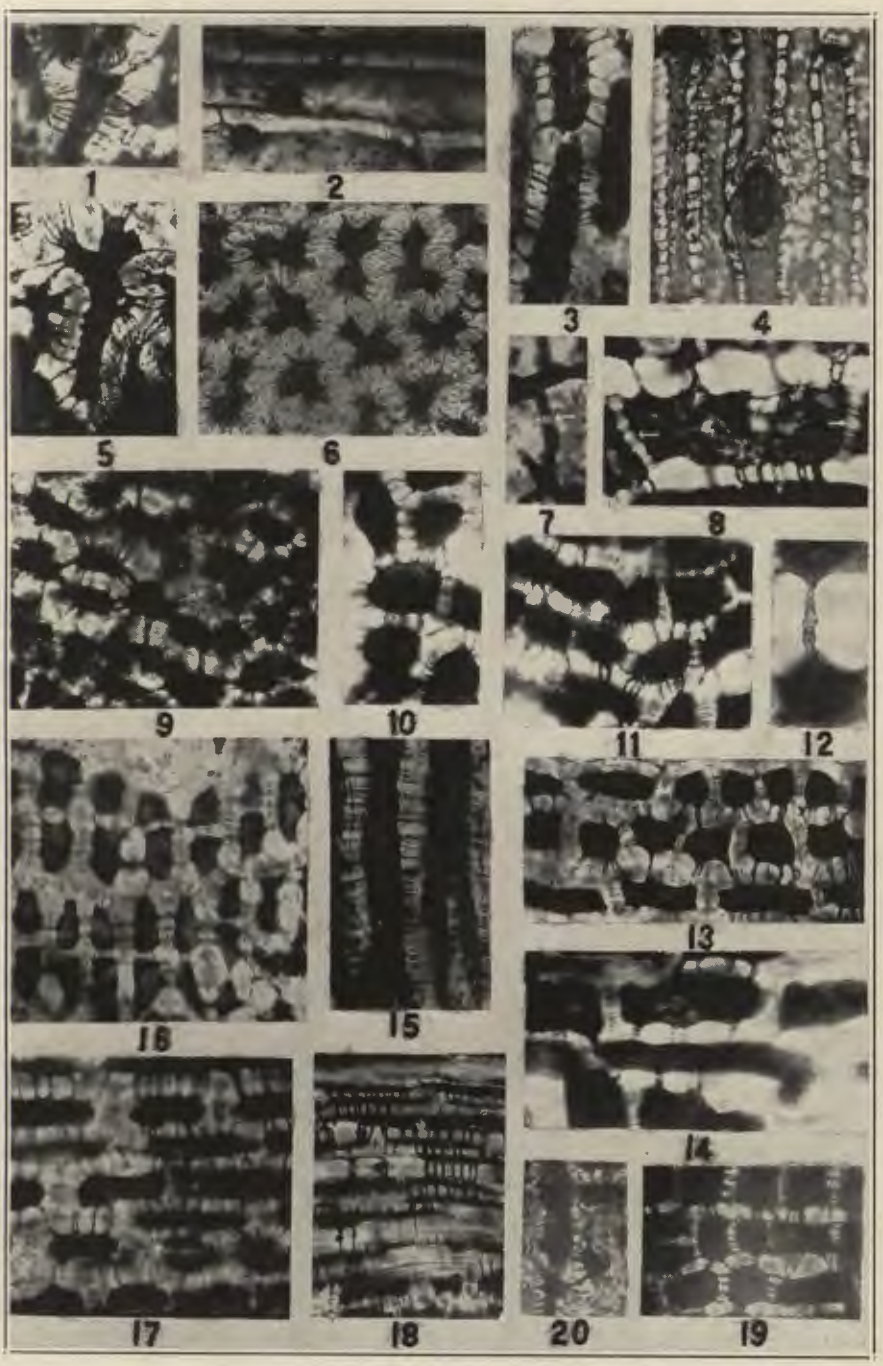
Fig. 17. Plasmodesms in the cortex of the stem of a tomato; x 320.

Fig. 18. Plasmodesms in the cortex of the common elder; x 200.

Fig. 19. Plasmodesms between the ray cells of an apple twig; x 320.

Fig. 20. Radial lengthwise section of an apple twig showing the plasmodesms between a ray cell and adjacent parenchyma cells at right angles to the ray cells; x 320.

PLATE I



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